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ENDOCRINES IN INVERTEBRATES

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The study of invertebrate endocrinology, although it provides interesting parallels to the hormonal activities in vertebrates, should be viewed not merely as an extension of endocrinological research to the invertebrates, but as a field presenting new questions of general importance. Among these is the problem of non-autonomous development of hereditary characters under the influence of diffusible substances ("gene hormones"), or the physiological significance of neurosecretion. It is with these and similar contributions which the study of invertebrates has made to endocrinology that this article is primarily concerned.

SEX HORMONES. Long before other hormonal activities became known in invertebrates the presence of sex hormones was discussed with respect to worms as well as molluscs and arthropods. However, there still is considerable controversy concerning the interpretation of the data available at present.

Evidence of the occurrence or non-occurrence of sex hormones in invertebrates has been furnished in different ways: 1, by the analysis of "parasitic castration," a phenomenon consisting mainly in a partial or total destruction of the gonads by parasites; 2, by experimental castration accomplished surgically or by irradiation, for from changes in secondary sex characters occurring after castration the presence of sex hormones is indirectly concluded; 3, by transplantation of gonads between the two sexes; 4, by regeneration experiments; 5, by assaying invertebrate material in vertebrates and vice versa (p. 403).

Among the *worms* the male gonads were found to control the development of the copulatory organs in turbellarians, for example, Planaria. Regeneration of these secondary sex characters failed in the absence of

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the anterior body region which contains the gonads, and in the case of parasitic castration (179). Similarly the differentiation of a secondary sex character typical for the annelids, the clitellum, seems to take place under the control of a male sex hormone. According to Hess and Bacon (202) this hormone originates in the seminal vesicles and not in the testes. For a discussion of other experimental data on this problem see Heumann (84).

Further evidence of an endocrine function of the gonads was obtained in *molluscs*. The hectocotylus, a secondary sex character of the cephalopods, depends in its development upon the presence of the gonads (163) and so do the accessory characters of the genital apparatus in the snail Littorina (115).

That sex hormones play a certain rôle in *crustaceans* appears now also probable, although only indirect evidence is available. Numerous cases of parasitic castration have been carefully analysed since the first observation of the phenomenon (64). In the crab Inachus, for instance, the parasites (Sacculina, fam. Rhizocephala) cause a varying degree of destruction of the gonads and consequently influence the secondary sex characters which show an outright dimorphism in the male and female hosts. Already existing characters disappear and are replaced to a certain extent by those characteristic of the opposite sex; i.e., the infected male becomes typically female in appearance. It is of importance that the alteration of secondary sex characters is not merely a return to the juvenile type, but an approach to the adult features of the opposite sex (177). In the majority of cases these changes toward the opposite sex concern the male, whereas the infected female in most cases loses only some of its own secondary characters. There are, however, a few indications that the female may assume a few characters more or less male (165). Although in all these observations the extent of the infection and the effects of it may vary considerably in regard to different hosts and various parasites (for a discussion of the extensive literature see 59, 102, 80), castration generally results in an alteration of the secondary sex characters. Interpretations of the findings are controversial (166, 67, 122, 178, 27, 102, 80); however, in the crustaceans an endocrine significance of the gonads seems undeniable. This view is supported by experiments in which sterilization of females by radium irradiation prevented the development of certain secondary sex characters such as the brood pouch in the isopod Asellus (74) or part of the cyclical secondary characters in the amphipod Gammarus (114). Similar conclusions as to the rôle of sex hormones in crustaceans were reached by various workers (10, 127, 128, 50, 56, 43).

Unlike the crustaceans, the *insects* responded with negative results to experimental castration or transplantation of gonads. After grafting gonads of the opposite sex into castrated caterpillars, Meisenheimer (121) observed no influence on the secondary sex characters of the adult moths. After surgical castration of larvae the respective external secondary sex characters developed fully in the resulting insects and the sexual behavior appeared to be unaltered, as described by a number of investigators (see 80).[2] In contrast to all these negative results, similar experiments in the Lepidopteran Cosmotriche (148), for instance, suggest a possible hormonal influence of the gonads on some, at least, of the secondary sex characters of this species (see also 53). Furthermore, an effect of this kind is clearly observed after parasitic castration. "Stylopization," i.e., castration by the parasite Stylops, first described in the bee, Andrena, by Pérez (see 189), results in changes of the secondary sex characters of both sexes. In infected males the color of the face (clypeus normally yellow) and the structure of the hind legs assume a female appearance. Similarly in a stylopized female, besides other changes, the pollen-collecting apparatus of the legs becomes almost as reduced as in the male and the color of the clypeus changes from its normal black to yellow. Most of the additional contributions to this problem are in accordance with Pérez' classical observations (see 102, 80). Thus recently again Rempel (151) found in Chironomus, parasitized by nematodes, the external genitalia "distinctly under the control of the sex cells, this control, as in vertebrates, being in all probability exercised through the medium of a hormone."

Mention must be made in this connection of the occurrence of gynandromorphs in which by direct cellular determination each part of the body develops independently of other parts (mosaics). Gynandromorphs should not be considered, as some authors have considered them, as contradictory to the assumption of a hormonal control of secondary sex characters, since different tissues may respond differently to the presence and concentration of hormones. The data available at present about "intercastes," i.e., intermediate forms between the normally well differentiated castes of ants caused by parasitic nematodes (190, 180), do not furnish conclusive evidence of possible participation of hormones in the development of insect castes.

Finally, the mutual relationship between the corpora allata (p. 395)

[2] I. W. Pfeiffer again found that, in grasshoppers, castration and grafting of gonads do not influence the development of secondary sex characters. (Personal communication.)

and the ovaries in adult insects (175, 204; p. 400) seems to speak in favor of a humoral rôle of insect gonads.

It thus appears that the assumption of an active rôle of sex hormones in insects is still rather inadequately supported, although it cannot be completely refuted. From the data available at present several possibilities are open for discussion. 1. Sex hormones may be present in the insect body, as was shown to be actually the case by Loewe and his co-workers (116, 117; p. 403), but they may not influence the secondary sex characters (104). Observations in parasitized insects tend to refute, however, this possibility, unless one prefers to accept Goldschmidt's (68) interpretation of parasitic castration as a type of intersexuality. 2. The negative results in most of the experimental work dealing with this problem might be explained by the fact that the operations were not complete or that, at the moment of castration or transplantation, the secondary sex characters had already been determined. This assumption is ruled out at least in the experiments of Geigy (62) who obtained complete castration by elective ultraviolet irradiation of the primordial germinal cells in the "egg" of Musca. There was no indication of an endocrine activity of the gonads, at least regarding the differentiation of secondary sex characters. 3. Sex hormones may be produced within the insect body in an organ other than the gonad itself. This is denied, for instance, by Rempel (151) but is suggested by the work of Iwanoff and Mestscherskaja (87) who, on the basis of transplantation experiments in Blatta, attribute to the fat body the formation of a sex hormone responsible for the maturation of the gonads as well as for the determination of the secondary sex characters. A second hormone, originating in the degenerating egg follicles, was claimed by the authors to infantilize the ovaries temporarily, i.e., for the period in which the cocoon is carried by the female. As similar effects are obtained with unspecific substances, the experiments as well as their interesting interpretation need further confirmation.

Thus in insects the state of affairs is still not clear and at present the question of a hormonal control of sexual functions cannot be answered satisfactorily, whereas in crustaceans, worms and molluscs the evidence available points towards an active rôle of hormones in the determination of secondary sex characters.

GENE HORMONES. It is known that in certain cases, mainly insects, hormonelike substances play a rôle in the development of hereditary characters. Some tissues of animals containing certain genes were found to produce and give off specific diffusible substances under the influence

of which the respective characters develop. These gene-controlled substances ("gene hormones," 109), when released from cells of the genotypes, may induce in animals which lack these particular genes a non-autonomous development characteristic for the donor cells. For reviews of these problems, so far mainly worked out by Kühn, Ephrussi, Beadle and their co-workers, the reader is referred to the papers of Ephrussi (58) and Plagge (147).

The action of gene hormones can be ascertained in various ways. One of these is by an analysis of insect mosaics. This was first done by Sturtevant (172) who in gynandromorphs of Drosophila melanogaster found, for example, the female organs to influence the color of the genetically male eyes. A second is by endocrinological methods, i.e., grafting of tissues between donors containing and hosts lacking a certain gene and vice versa, or by application of the gene hormones themselves through feeding, injection or the addition to organ anlage in vitro.

Characters developing by means of gene hormones are, for example, certain eye colors. Thus in the meal moth Ephestia there exists, except in the black-eyed wild race (a^+a^+), a mutant with red eyes (a a). If into a-larvae the testes of wild-type larvae are grafted, the resulting adults exhibit normally pigmented, that is, dark eyes instead of the expected red ones. One must conclude, therefore, that the implanted testis furnishes a substance which in the host modifies the development of the genetically determined eye pigment. In the red-eyed mutant the agent responsible for the formation of normal, i.e., a^+ or wild-type eye pigment apparently is lacking as a consequence of a genetic constitution altered with respect to one gene (a). This deficiency can be overcome, however, as the mutant retains its ability to respond to the diffusible agent. In Ephestia this substance, called "a^+ hormone," is produced in addition to the gonads also in the brain and ventral cord (150), in the fat body and in the hypodermis (124). Other non-autonomous characters undergoing similar somatic changes under the influence of a^+ hormone are the color of the skin, of the testis and of the brain. Thus an a-testis implanted into an a^+ host assumes the phenotype of the wild race under the influence of the a^+ substance circulating in the body of the host. Similarly certain eye-color characters in Habrobracon, a wasp parasitizing on Ephestia, were found to be non-autonomous (191).

In Drosophila, in view of the experimental results obtained, two eye-color hormones have been postulated, one for the character "vermilion" (v^+ hormone) and one for "cinnabar" (cn^+ hormone). Both substances

are produced in the eyes and in the Malpighian tubes, while the fat body only furnishes v^+. The v^+ substance must be considered as an active precursor in the formation of the closely related cn^+ substance. Both substances are contained in the a^+ substance of Ephestia and they were also found in corresponding substances obtained from a number of insects (112) as, for example, the fly Calliphora, the moth Galleria, and even from the crab Uca.

Gene hormones are thus neither species- nor genus-specific. It is of interest that in Drosophila pigment formation in eye anlagen could also be observed in vitro. If v^+ hormone was added to the culture medium, anlagen of organs lacking v^+ gave a similar response as in vivo (69). The gene hormones, found to be present in the body from early stages, seem to be furnished by the different organs in various amounts and at different times. The formation was found to begin in Drosophila eyes, for instance, 40 hours after puparium formation (47). The concentration in the lymph of the wild type is higher in pupae than just before puparium formation. The influence of the gene hormones is exerted at definite times during development ("sensitive" or "effective" periods). Gene hormones are maternally transmitted to the F_1-generation. They are stored in the organs of formation; their production is greatly influenced by nutrition (174). Quantitative studies indicate that a^+a^+ cells produce the same amount of hormone as a^+ a cells. Within certain limits the reaction obtained after injection of gene hormones is proportional to the concentration. The substance is not released from an organ, for instance a wild-type eye, before the fulfilment of the organ's own requirements ("priority effect").

Gene hormones can be extracted from the organs and, in Drosophila, they can be separated from the pupation hormone (p. 398). As they have been purified to a certain extent, some of the physico-chemical properties are known. The gene hormones are heat resistant, soluble in water, less soluble in alcohol, acetone, etc.; they are dialyzable. Toward acids and alkali they react similarly as the pupation hormone (p. 398). A substance having the chemical and physiological properties of the $v^+(a^+)$ hormone was found to be synthesized by certain bacilli in the presence of tryptophane (173, 42). This lead Butenandt, Weidel and Becker (42) to the conclusion that the $v^+(a^+)$ substance is probably identical with kynurenine, a derivative of tryptophane, which has a rather specific effect on the eye coloration of Drosophila and Ephestia. Whether this substance will prove, on further study, to be a "gene hormone" or rather a chemical precursor of the eye pigment cannot be

decided at present. For other interactions between hormones and genetical constitution see (72) and (141).

COLOR CHANGE. As in vertebrates, there occur in invertebrates two types of pigmentary reactions: 1, a morphological color change consisting in the formation and destruction of pigments, and 2, a physiological color change caused by the more or less rapid expansion or contraction of the chromatophores, which in most cases is brought about by the migration of pigment within the cell. The physiological color change is of most interest here, since it is controlled to a large extent by hormonal (neurohumoral, 132) factors.

1. Crustaceans. The group of invertebrates best known with respect to an endocrine control of pigment migration are the crustaceans and among them particularly the decapods. Koller (98, 99) was the first to find in Crago (Crangon) vulgaris that under certain conditions blood transfusions result in distinct reactions of the pigmentary system. He therefore assumed that a humoral agent circulating in the blood plays an important rôle in color adaptation. Later Perkins (133) in Palaemonetes and Koller (100) in Crago simultaneously described the eyestalk as the place of formation of this substance or substances (p. 392) which now are mostly referred to as "crustacean chromatophore activators" or "crustacean chromatophorotropic hormones or principles." The chromatophore activating material is produced within the eyestalk in the sinus gland, formerly called blood gland (78), as can be shown by injection of extracts or by implantation of the sinus gland into the abdomen of eyestalk-amputated crustaceans (33). The glandular structure of the sinus gland and its intimate relation to the blood system characterize it as an endocrine organ. The rôle of a second endocrine gland (X-organ) in the eyestalk is still unknown (76).

Generally humoral agents, if present in sufficient concentration, bring about color change either by preventing diffusion or by causing contraction of pigment on the one hand, or on the other hand by permitting, if absent, expansion of pigment. The same agent may, however, have the opposite effect, depending upon the species and its pigmentary apparatus. In other words, expansion of the pigment may indicate the absence of chromatophorotropic hormone in the blood (Palaemonetes, 133), or it may be a sign of its presence (Uca, 44). In most of the cases studied (for exceptions see 100, 167, 35, 198, 36, 199) there seems to exist no humoral agent acting antagonistically to the eyestalk substance (32). Consequently one phase of pigmentary activity (expansion or contraction) is hormonally induced, and the reverse phase results

passively on the basis of a property inherent in the pigment cell itself. Different chromatophores may differ in their responses to the chromatophorotropic hormones and show a certain independence from one another in a given animal (29, 30, 199) and they vary in different genera, although one type of chromatophore may be chiefly responsible for the outcome, as for instance in Uca. Pigment expansion may also be only partial or there may be in the same animal locally different responses (101). The combined reactions of the various kinds of chromatophores in response to the release of the chromatophorotropic hormones into or its disappearance from the circulation result in a state of distribution of pigment characteristic for the different species. Finally a certain degree of inconsistency of pigmentary reaction (1) and possible responses to stimuli other than the specific hormones (pp. 391–392) represent certain difficulties in the evaluation of experimental results.

In the study of crustacean color change several ways of experimental approach may be used. 1. Under different conditions of light, background, time of day, and so forth, the chromatophores show expansion or contraction. On a white background, for example, the chromatophores in Palaemonetes exhibit contraction due to the presence of eyestalk hormone (28). Certain species, for instance Uca, show a diurnal rhythm of pigment migration, considerably uninfluenced by environmental conditions, particularly by constant darkness (184). This rhythm, the mechanism of which is still unexplained, is attributed to a periodic release of hormone and not to its cyclic exhaustion and elaboration. 2. In the great majority of cases the color change in crustaceans is disturbed by the operative removal of both retinae or the coating of the eyes, as pigment migration takes place in accordance with stimuli received through the eyes (30). In a brachyuran such an operation causes expansion of melanophores as in lower vertebrates. This indicates that after eye excision, chromatophorotropic hormones are continuously released, thus also disturbing, at least temporarily, the diurnal rhythm (171, 6). The ventral half of the eye, as in certain fishes, is the decisive factor (79). Heterologous stimuli of the optic nerve, i.e., electrical stimulation or slight cauterization in eyestalk-amputated animals, or cutting of the optic nerves, have the same effect as has light on normal eyes, in a similar way as in the frog. 3. Results completely different from those just outlined are obtained after amputation of the entire eyestalks, sometimes still, but incorrectly, referred to as "blinding." Here the source of the chromatophorotropic material

is removed with the effect that, independently of the color of the background or of light conditions, for instance, the red pigment in Palaemonetes is permanently expanded. 4. Chromatophorotropic hormone can be added to the circulation by injection of organ extracts into either normal or eyestalk-extirpated crustaceans or by feeding hormone containing tissue (100). If the source of eyestalk extract is the same species or even the test animal itself, the coloration obtained is that characteristic for the presence of hormone in the blood of an intact animal (133). Therefore, the result of such an experiment as this is predictable, provided that the normal behavior of a given species is known. Qualitatively the same results are obtained, as a rule, after reciprocal injections, as for instance, of eyestalk extract from Palaemonetes injected into Uca. Tests among at least 30 different crustaceans showed that frequently eyestalks exhibit more potencies than the donor's pigmentary activity would require. Similarly successful were tests with organ extracts from sources other than crustaceans, such as corpora cardiaca, cerebral and frontal ganglia of insects (36; see also 77, 78, 90), central nervous system of Limulus (197; see also 205), neural gland of ascidians (5; pp. 401–402), sheep pituitary (p. 402).

Thus there exists no specificity regarding the efficacy of the active principles in crustacean color change. This interspecificity is also shown by the fact that crustacean eyestalk hormones act upon vertebrate chromatophores (p. 403).

As to the specificity of the hormone producing organ in crustaceans, there are, in addition to the sinus gland, few tissues to which similar effects on pigmentary distribution have been ascribed (8, 97). The "rostral organ" of Koller (100; see also 15, 32) acting antagonistically to the active material of the eyestalk seems to be confined to very few species (135, 106). Brown and co-workers (35, 198, 199) found part of the circumesophageal connectives (commissural gland) of Crago and Palaemonetes yielding a substance dispersing the dark pigment of the telson and uropods of Crago, and while white pigment is dispersed by sinus gland extracts it is contracted by an agent present in the central nervous system (36). There is indication for the presence of two different hormones in the commissural ganglia (199).

Few substances from other sources than those just mentioned are known to act upon crustacean chromatophores. In some species expansion of pigment is caused by adrenaline (15). The interesting pigmentary reactions observed after injection of distilled or tap water (8) are probably due to osmotic changes in the animal. This suggests the

possibility that the chromatophorotropic hormones affect the chromatophores by regulating primarily the salt or water balance of the animal. The occurrence of a chromatophore activator in species lacking chromatophores would thus be explained (78, 169). Sea water injections may —possibly by a dilution of hormone present in the blood—or may not affect the state of pigment distribution. Further influences affecting crustacean color change are temperature and such ions as Li, Na, K, Ca, which cause slow and irregular responses (15). A number of other agents, as, for example, different drugs, electrical current, growth substance of plants (133, 15, 129), have been found negative.

In crustaceans, eyestalk extracts show some effects other than those produced upon chromatophores, but the exact origin of the responsible substances within the eyestalk or their relation to the chromatophore activators is not known in all these cases. One of the effects concerns the pigmentary adaptation of the compound eye. In animals kept in the dark, after injection of eyestalk extracts from light-adapted specimens, a migration of the distal and the reflecting retinal pigments takes place. The resulting position is such as that typical for a crustacean kept in the light (95, 185, 49). In the dark there occurs not only diminished release or none, but apparently either less or no synthesis of the substance influencing the retinal pigments. This hormone, which is believed to be different from the chromatophore activators (78), is not genus-specific. Furthermore it was found that there exists, for instance, in Cambarus a diurnal rhythm of retinal pigment migration which continues for some time in specimens kept in the dark. This has been explained (208) by a regularly changing activity of nervous centers inhibiting the hormone release.

Other influences attributed to the eyestalks concern the calcium metabolism (101; but see also 203), and the rhythm of the heart (183) and the musculature (91) of crustaceans. Eyestalk removal was found to cause acceleration of molting and increase in body size (34, 9, 203, 206). A favorable effect of the presence of the eyestalks or their extracts on the viability of crustaceans was claimed by Brown (31, 34), but this was not confirmed by Kleinholz (203).

When summarizing the data on the activity of the chromatophorotropic principles in the crustaceans two explanations offer themselves (5). 1. According to the unitary theory the organization of the various pigmentary effectors is responsible for the different reactions caused by one common eyestalk hormone. 2. The multiple theory claims the presence of more than one chromatophorotropic hormone in crustaceans;

at least two active substances are postulated by Kleinholz (96) to explain the known facts. A first attempt toward a physiological and chemical differentiation of two such principles within the sinus glands of seven different species was made by Brown and Scudamore (37; see also 198, 199). However, no final answer as to the number of active principles can be given so long as their chemical constitution remains unknown.

Some quantitative estimations regarding a chromatophore activator of Palaemonetes (4) suggest the following conclusions: *a.* In black-adapted animals a small quantity of hormone is present in the eyestalk. Incident light, regardless of the background, steps up the formation of hormone as compared with darkness. *b.* If, in addition, the light is reflected from a white background, a maximal hormonal release into the blood results and an increased rate of hormone synthesis takes place. The rate of color change was found approximately halved after amputation of one eyestalk (30, 45).

A method for the standardization of a crustacean chromatophore activator was devised by Abramowitz and Abramowitz (8) who used as criterion the reaction of the black chromatophores of eyestalk-extirpated Uca pugilator. The minimal amount of hormone detectable in such assays is 0.000016 γ. The thresholds of melanophore responses vary from animal to animal and "the degree and the duration of melanophore expansion are exponentially proportional to the dosage injected."

The relative effectiveness of extracts from the sinus gland and from the commissural gland (p. 391) differs with respect to different pigments (199).

The properties of the chromatophorotropic hormones may be summarized as follows. 1. Originating in distinct regions of the body the hormones, by way of the body fluid, reach the effector organs upon which they exert specific effects. 2. The action of the chromatophorotropic hormones is inter-specific (p. 391). 3. Solvents for the hormones are water (133), absolute ethanol, and methanol; they have been found insoluble in ethyl ether, chloroform, acetone, and benzine. 4. They are resistant to digestive enzymes (101), to boiling (133, 36) and to short boiling with alkali or acid, to cold temperatures (129), and to desiccation (135, 36), but they are destroyed by oxidation. 5. The hormones are readily adsorbed (3). 6. Although purification of eyestalk extracts has been performed by Abramowitz (7), the chemical constitution of the chromatophore activators, probably nitrogenous bases, is not yet known and the question of their identity with the intermedin of the vertebrate

pituitary must therefore be left open. Both materials show such close similarity in their physical and chemical properties (solubility, etc.) as well as in their physiological behavior, that they yield reciprocal results, provided they are administered in proper doses. Crustacean eyestalk extracts act upon vertebrate chromatophores (103, 125, 134, 107), and sheep pituitary and neural gland of ascidians influence crustacean color change (2, 5).

2. Insects. In a few groups of insects a morphological as well as a physiological color change has been observed. The best known example is the phasmid Dixippus morosus which, like some crustaceans (p. 390), shows a diurnal rhythm of coloration (184). Colored illuminated backgrounds influence the coloration of Dixippus (65, 11, 149, 88), but the effect disappears when the lower half of each eye is coated. Such animals soon become dark because of expansion of the pigment (physiological color change). Later, in addition, a formation of pigment takes place (morphological color change). Similar behavior is characteristic of an animal kept constantly on a dark background. Body regions from which the blood supply is temporarily cut off by ligatures become pale and remain so until circulation is restored. Decapitation results in paling of the whole animal (88). Transplanted pieces change their coloration simultaneously with the host (89).

From such observations and from the lack of an innervation of the cells responsible for the color change hormonal control of the physiological as well as the morphological color adaptation was concluded. Absence of the hormone in the body fluid results in concentration of pigment and in cessation of pigment formation. The source of this endocrine substance is in the head region of the animal. It is also known that the corpora allata (p. 400) play a rôle in the color responses of Dixippus (79, 139, 141), but this rôle is not yet fully understood. Denervation of the corpora allata resulted in unusual color patterns (morphological color change); reimplantation of corpora allata into allatectomized animals caused blackening of the hypodermis in the neighborhood of the implant. On the other hand, extirpation of these organs as well as of the corpora cardiaca (p. 397) had no influence on the color change in Dixippus (11, 139).

In addition to light, humidity also was found to cause pigmentary changes through hormonal action in Dixippus. On the other hand, local color reactions, brought about by temperature changes, distilled water, ether, etc., are not under hormonal control.

The effect of extracts from insects, such as Dixippus, on the chromatophores of crustaceans has already been mentioned (p. 391).

The possibility of hormonal control of the morphological pigment adaptation in certain pupae of Lepidoptera (25, 26) and of the physiological color change in the air-sacs of Corethra larvae (118) has been discussed by Giersberg (66) and others.

Concerning possible hormonal factors in the color adaptation of cephalopods see Sereni (164) and p. 401.

HORMONAL CONTROL OF INSECT DEVELOPMENT. Since Kopeć (105) showed for the first time that pupation in an insect (Lymantria) is brought about by a humoral agent, the existence of hormones involved in molting, pupation and metamorphosis has become a well established fact. The presence or absence of such hormones was demonstrated in a comparatively simple way by *a*, blood transfusions; *b*, by interruption of the circulation by means of ligatures; *c*, by injection of various extracts, and *d*, by transplantation of certain organs. In experiments of this kind the "critical period" was found to be of importance, i.e., a distinct phase preceding ecdysis, pupation, or emergence of the adult, which represents the time of hormone release (p. 398). With such methods an endocrine control was ascertained with respect to molting in Lepidoptera (18, 146) and Hemiptera (192), and to pupation in Lepidoptera (105, 110, 146) and Diptera (60, 18, 19, 123, 16, 52); see also (63). Similarly a factor governing the differentiation of the imago was found to originate in the anterior body region of Lepidoptera (71, 18, 142, 143, 209), of Diptera (19, 20; see also 92) and of Hemiptera (192). The hormonal nature of this factor for which good evidence has been furnished by the work of Piepho on Lepidoptera (142, 143), and others, has been questioned, although not disproved for Drosophila (21).

In recent years, with the development of a more delicate technique, viz., extirpation and implantation of glands in various developmental stages, more detailed results were obtained. At present the interest centers around such questions as the analysis of different developmental steps, the origin of the hormones involved in these partial reactions, the chemical nature of the hormones and their mode of action in correlation with the effector organs. In an evaluation of the data now at hand it should be borne in mind that they refer to representatives of different types of insect development, i.e., Holometabola and Hemimetabola.

So far mainly two organs of internal secretion, the corpora allata and the cerebral ganglion, are known to play an important rôle in the molting and metamorphosis of insects. Kopeć (105), and later Kühn and his co-workers (110, 142, 146), demonstrated by two procedures that

in Lepidoptera the supraesophageal ganglion (brain) furnishes a substance causing pupation. 1. Extirpation of the brain after the last larval molt prevents pupation, provided that the operation is performed before the critical period. 2. Reimplantation of a brain into such an animal restores the capacity for pupation. A possible rôle of the frontal ganglion in pupation has been discussed also (23).

In the hemimetabolous Hemiptera the development is characterized by the occurrence of a number of molts, the last of which is coupled with metamorphosis and leads to the adult stage. Here a part of the nervous ganglia produces a "molting hormone," as was conclusively demonstrated by Wigglesworth (195). If, in the bug Rhodnius, the central mass of the protocerebrum from a donor in the critical period is implanted into decapitated nymphs, molting occurs. Other parts of the central nervous system, the corpora allata, the fat body, etc., give negative results.

Wigglesworth showed in Rhodnius, in addition to the molting hormone, the action of an antagonistic principle, the "inhibitory hormone" which originates in the corpus allatum (192, 195). There is good evidence that this factor, in all but the last larval instars, prevents metamorphosis and favors the occurrence of further nymphal molts. Thus in collaboration with the molting hormone the inhibitory hormone controls the rate of development. The experimental data obtained not only in Hemiptera and Orthoptera, but also in Lepidoptera, are in agreement with this assumption.

1. In Rhodnius (Hemiptera) removal of the head during the critical period, i.e., excluding the inhibitory factor just before its release into the circulation (p. 399), causes metamorphosis to occur earlier than normally. Extirpation of the corpora allata in the third or fourth nymphal instars of Dixippus (Orthoptera) decreases the number of subsequent molts and thus causes accelerated maturation (137). Similarly in early larval instars of Bombyx (Lepidoptera) allatectomy leads to suppression of molts and to precocious pupation and results in normal adults (24).

2. Removal of the corpora allata in the last nymphal instar of the Orthopteran Melanoplus (136) or in the last larval instar of the Lepidoptera Bombyx (24) and Ephestia (110) has no influence on the final development. In these cases no inhibitory factor is active and the removal of its source, therefore, does not show an effect.

3. Implantation of corpora allata from a young caterpillar into one which is ready to pupate, i.e., adding the inhibitory factor where it is

normally absent, prevents the onset of metamorphosis in certain moths (110, 146, 144, 24). In grasshoppers (140) or bugs (194) the implantation of corpora allata may thus also cause several supernumerary molts.

In this way the function of the corpora allata appears to be "inhibitory" with respect to the development of adult characters in representatives of three orders of insects, viz., Hemiptera, Orthoptera, Lepidoptera.

In a representative of the Diptera, Drosophila melanogaster, Hadorn (72) proved that puparium formation is brought about by an agent originating in the ring-gland (Weismann's ring). Puparium formation, which is greatly retarded or fails to occur in lethal larvae of the mutant lgl and in certain hybrids, can be accelerated by implantation of genetically normal ring-glands. This effect cannot be brought about by brain implants. Furthermore, within certain limits puparium formation in normal larvae takes place the sooner the more mature ring-glands (from one to three) are implanted (73). This rôle of the ring-gland was confirmed for Calliphora by transplanting the organ into posterior larval fragments (16). Correspondingly, extirpation of the gland or section of its nervous connections in Calliphora prevents puparium formation and arrests the growth of the imaginal buds (40). In Drosophila, however, the ring-gland does not influence imaginal development (73). Morphologically the ring-gland is the homologue of both corpus allatum and corpus cardiacum. The histological appearance of the two types of glandular elements in normal as compared with lethal Drosophila larvae (154; p. 398) suggests that the larger gland cells which now are believed to represent the corpus cardiacum (201; cf. 39) are the possible source of the "pupation hormone."

To summarize: In three (Hemiptera, Orthoptera, Lepidoptera) of the four orders of insects studied the corpora allata are known as the source of a hormone inhibiting imaginal differentiation. In the Diptera a principle causing puparium formation is assumed to be furnished by a glandular tissue which represents the corpus cardiacum. The endocrine rôle of the supra-esophageal ganglion has been demonstrated in two orders, i.e., in Lepidoptera where it controls pupation, and in Hemiptera where it furnishes a hormone, that, in coöperation with the inhibitory factor (p. 396), controls molting.

Whereas the glandular character not only of the corpora allata, but also of the corpora cardiaca has been known for some time (192, 137), the concept of an endocrine rôle of the nervous system has been accepted with considerable hesitation, in spite of the fact that the occurrence of

nerve cells with an undoubtedly glandular character has been demonstrated in invertebrates as well as in vertebrates (155). That, in the insects studied, such neuroglandular cells are actually the source of the hormones found in the physiological experiment is strongly suggested by the following histological findings: neurosecretory cells occur not only in the larval brain of Lepidoptera (51), such as Ephestia, but also in Rhodnius where they are located in the pars intercerebralis (79), i.e., the very region of the nymphal brain yielding the molting hormone in the experiments of Wigglesworth (195; for further literature on neurosecretory cells in insects see 153, 200; concerning the endocrine rôle of the central nervous system in color change, see p. 391).

The data just reviewed may seem to be discordant. It should be kept in mind, however, that insect development is a complex mechanism with more factors involved than those here outlined, and that the various orders differ from one another to a considerable extent. Furthermore, it is probable that additional endocrine factors will be discovered which will lead to a better understanding of a field that has, as yet, only been touched upon. Nevertheless, some generalizations can be made now with regard to the properties and the mode of action of the hormones controlling insect development.

1. It seems rather certain that more than one principle is involved in the different steps of insect development. To what extent these substances differ from one another can hardly be decided so long as their chemical constitution is still unknown.

2. Extracts made from and tested in Calliphora led to a certain purification of the pupation hormone and to its separation from the a^+ substance (p. 387), with the result that some of the properties of the pupation hormone are known (16). It is soluble in water, alcohol, butanol, acetone, etc.; insoluble in chloroform, petroleum ether, etc.; resistant to heat and acids but not to alkali. It is readily dialyzable.

3. The hormones regulating insect development are not specific with respect to the genus (193, 73, 143), or even to the order (16).

4. The proper action of the hormones depends on the state of the glands which furnish them, as well as on the state of the reacting organs and tissues. Hormones are produced and released only by mature glands. Thus in the mutant lgl of Drosophila, for instance, the inability to pupate (p. 397) was found to be due to a genetical deficiency to develop fully grown ring-glands which lack the power to produce a sufficient amount of pupation hormone (154). The release of hormone takes place gradually during the "critical period" (p. 395) which, in

Calliphora, for example, is 8 to 12 hours before puparium formation (16). In a case where, as in Rhodnius, two hormones interact, the factor inhibiting metamorphosis was found to be given off later than the molting hormone (192).

5. Regarding the reaction of the effector organs to the hormonal stimulus, two points are of importance. *a.* The reacting organs (hypodermis, Verson glands, etc.) must be in a physiological state of preparedness in order to guarantee proper responses (19, 146, 73, 145). *b.* Within this limit the reacting material exhibits a considerable independence. Grafted organs are capable of undergoing developmental changes simultaneously with their host and to a certain extent irrespective of their own ages. This may lead to supernumerary molts or to accelerated development of the implants (119, 20, 111, 195). The longer before the critical period the stimulus occurs, the less sensitive is the reacting material to a given hormone concentration (16). The response to the pupation hormone is characterized by well defined steps, viz., onset of mitoses, separation of larval cuticle from hypodermis, secretion of exuvial fluid. The latter two phases may be suppressed if the hormone supply is insufficient (111). But once a sufficient stimulus for pupation or for imaginal differentiation has been received, completion of the process may take place independently of the subsequent presence or absence of the stimulating hormone (145, 20).

6. The action of the hormonal factors governing insect development is to a certain extent quantitative. Small amounts of hormone may yield incomplete metamorphosis (193) or partial pupation (110, 111, 142, 16; see also p. 393). In such cases certain regions of the hypodermis prove to be more sensitive to low hormone concentrations than others, an observation which has been connected with the important rôle oxygen plays in the differentiation of insect tissues (21, 16).

7. Various environmental factors interact with those of hormonal nature in the regulation of insect development. In the mealmoth, Ephestia, the release of the pupation hormone can be inhibited by low temperatures. The resulting "permanent larvae" pupate when brought back to room temperature (E. Caspari, personal communication). Combinations of pupal and adult characters may be obtained in individual silkworm pupae by the application of temperature gradients (209). For other examples see, for instance, the papers of Mellanby (123) or Bodenstein (196).[3]

[3] Regarding hormonal interactions between ovary and molting rhythm in isopods (crustaceans) see Haemmerli-Boveri (74).

Aside from their rôle in insect development *additional endocrine functions of the corpora allata* may be briefly discussed. Their presence is necessary for the attainment of sexual maturity in some species (193, 175), but not in others (137).[4] In the grasshopper, Melanoplus differentialis, the corpora allata are necessary for the deposition of yolk and for the production of secretion by the oviducts. After reaching the proper stage of maturation the oviducts respond with secretion to the presence of corpora allata, irrespective of whether these glands are grafted or in situ, from males or females, from nymphs or adults (136, 204). Larval ovaries transplanted from Drosophila melanogaster into Drosophila funebris fail to develop yolk unless transplanted together with melanogaster-ring-gland (207; p. 397). Considering the results of Pfeiffer (136) it is possibly the allatum-component of the ring-gland which furnishes a substance necessary for the egg development of Drosophila melanogaster. Whether a species-specificity of a hormonal action really exists to such a degree needs further investigation. The corpora allata influence color change (p. 394), growth regulation (119, 141, 22) and regeneration (140). Removal of these glands in an early developmental stage causes degenerative processes in certain tissues such as the fat body, the musculature and the nervous system, as well as abnormal growths interesting for their similarity to certain tumors in vertebrate pathology. Reimplantation of the glands restores normal tissue growth (139). Actions of the kind described suggest that the rôle of the corpora allata consists in a general influence on metabolism (140), but the question is how far this concept may explain all of the special effects attributable to the glands.

The versatile character of the functions of the corpora allata in influencing color change, sexual maturation, regulation of growth, etc., might suggest a comparison of this gland with the hypophysis of the vertebrates. Although the concept is highly interesting, it should be kept in mind that a homology between the two organs should, by no means, be constructed from a comparison of this kind.

Except for the corpora allata, the corpora cardiaca (pp. 395, 397; see also 136), and the cerebral ganglia there occur in insects a few organs of

[4] The honey bee represents a special, although still somewhat controversial, case. Sexual maturation of the queen as contrasted with the worker bee is known to depend on the special food (royal jelly) provided during larval life. In this royal jelly an active material has been found which has a certain gonadotropic activity as demonstrated by its effect on the reproductive system of rats (85) and of Drosophila (176).

possible endocrine nature: the "pericardial glands" and the "ventral glands" (139, 141), the fat body (p. 386) and the silk glands (Umeya, see 80). The Verson glands, oenocytes, and synoenocytes are now believed not to belong in this category of organs (111, 80).

In addition to the reported data there are a number of more or less well established cases of hormonal actions in various invertebrates. Thus, for instance, the *internephridial organs* which form a part of the nephridia of the marine worm Physcosoma lanzarotae, show a striking similarity to the interrenal body of the vertebrates (81). Total extirpation of these glands leads to disturbances which resemble those of adrenocortical insufficiency in man (Addison's disease) and, within a few days, to death of the operated worms. Reimplantation of internephridial tissue or parabiosis with a normal animal brings about recovery. The internephridial cells exhibit various stages of a secretory cycle and their granular inclusions are similar to those of the adrenal cortex. These data do not hold true for Physcosoma japonicum, a species which survives total extirpation of the nephridia for more than two weeks (102).

Regarding hormonal activities in molluscs mention has already been made of the possible endocrine significance of the gonads (p. 384). Further organs of internal secretion are in all probability the *posterior salivary glands* and the branchial glands of the cephalopods (164, 126; see also 94). Similarly "purpurin" from the hypobranchial glands of the snail Murex (55) and a pressor substance related to "adrenin" in the mantle of Purpura (152) have been described as substances of hormonal character.

Further examples of supposedly endocrine functions which, however, before they can be included as true hormonal activities need further investigation, are found in the works of Hanström (80) and Koller (102).

In *tunicates*, the forerunners of the vertebrates, two organs show homologies with endocrine glands of the vertebrates. 1. The *endostyle* is commonly considered as a primitive thyroid. This gland, however, has no effect on amphibian development (168), and the acceleration of ascidian metamorphosis by vertebrate thyroid (181) is probably not due to thyroxine (70). 2. The *neural gland* (subneural gland) of the ascidians corresponds to the posterior lobe of the vertebrate pituitary. This is demonstrated not only by anatomical considerations but by pharmacological assays. The neural gland contains the oxytocic principle of the pituitary (tests with uterus of the rat and the guinea pig; 41, 12). The pressor effect is obtained upon injecting neural gland ex-

tracts intravenously into cats (12). A chromatophore-expanding action may be observed, for instance, in the frog (12, 57). There is no gonadotropic principle in the ascidian neural gland (17). The functions of both these glands in the tunicate organism are still unknown; the neural gland possibly regulates, through the oxytocic principle, the discharge of the sexual products (86).

An extensive literature (187, 102, 80) deals with the mutual *relationships between invertebrate and vertebrate endocrines.* Whether some of the invertebrate hormones are identical with known vertebrate hormones, cannot be decided so long as the chemical constitution of invertebrate hormones is so imperfectly known. However, irrespective of their chemical relations, vertebrate hormones are known to exert an influence on invertebrates and vice versa.

The response of invertebrates to *thyroid*, as tested by a number of investigators, is discussed by Schneider (156), with the conclusion that in protozoans and in fertilized eggs of several invertebrates "thyroid apparently accelerates both anabolism and katabolism resulting in an increased rate of cell division and excretion. Thyroxin, on the other hand, seems to affect only the katabolic processes in the cell, hence the accelerated rate of excretion and the slightly depressed rate of cell division." Regarding an influence on the development of arthropods the data are ambiguous, but at least in one carefully studied case a clear effect was observed. Thyroid-fed flour beetles (Tribolium) show acceleration of larval development and increase in growth. In a diminishing degree this effect is transmitted by the female to several generations fed on thyroid-free diet (157–161).

Parathyroid (Parathormone Collip-Lilly) is said to have a certain influence on the calcium metabolism of crustaceans (131; see also p. 392), but there is as yet no definite proof for a specific action of *thymus* on an invertebrate organism.

Growth, molting, and regeneration in isopods (Asellus) are reported to be considerably accelerated by *pituitary* extracts (75). Anterior lobe shows a gonadotropic influence, viz. accelerated maturation of eggs, in the polychete worm Lycastis (Feuerborn, see 80). Posterior pituitary is said to cause maturation in female insects (87). Color change in crustaceans is brought about by intermedin from vertebrate hypophysis (p. 391).

The *adrenal cortex* was found to exert a favorable influence on growth rate, maturation, and fertility of crustaceans (82, 46) and on the growth of snails (83). The effect of *adrenaline* on protozoans (186), i.e., an increase in the viscosity of the cytoplasm which is correlated with the calcium content of the medium, is of interest in view of general cellular physiology. Furthermore, adrenaline influences the vascular system of annelids (61) and it produces hyperglycemia in annelids, molluscs, and crustaceans (120), an effect which is considered by some authors as unspecific (93). Regarding the effect of adrenaline on color change see p. 391. As in vertebrates, adrenaline increases the tone and contractility in the musculature of worms, arthropods, and molluscs, causing such reactions as acceleration of the heart rhythm or, in cephalopods, pigment expansion (164, 13, 130, 108, 182).

There appears to be no known case of a specific action of vertebrate *gonadal extracts* on an invertebrate.

Insulin is said to have an influence upon the metabolism of protozoans, comparable to that in the mammalian organism (38). The chromaffinity found in certain ganglion cells of the leech (see below) disappears under the influence of pancreatic hormone (Poll, see 187).

Mammalian *secretin* stimulates the hepatopancreas of Octopus and an analogous substance extracted from this mollusc acts on the dog (113).

Otherwise comparatively few data suggest an effect of invertebrate endocrines upon vertebrates. The close similarity between the *chromatophorotropic hormones* of crustaceans and lower vertebrates has already been dealt with (p. 391). Furthermore, *estrogenic substances*, tested in castrated mammals, were found in almost all groups of invertebrates: protozoans (170, 14), coelenterates (162), worms (170), echinoderms (170, 116, 54), and molluscs (170). With few exceptions all results recorded for arthropods were positive, i.e., for several representatives of insects (170, 116, 117, 162), spiders (170, 162), scorpions (170), and crustaceans (54). See also footnote, p. 400. The seminal vesicles of castrated rats are said to become enlarged after injection of macerated seminal vesicles from earthworms (202). Finally, *adrenaline*, or at least a closely related substance, from invertebrates such as protozoans, annelids (chromaffine nerve cells), molluscs and insects (187, 188) was tested in vertebrates with positive results. Regarding tunicates see pp. 401–402.

The results of the experiments of which examples have been quoted above should be viewed critically. It must be kept in mind, for instance, that when feeding endocrine substances or adding them to culture media, the amount actually taken up by the animals is difficult to control. In some cases these substances are known to be quickly decomposed within the test animal before they can exert their influence, and the effects observed may be due to their nutritional rather than to a specific hormonal effect. A depressing action, on the other hand, may not rarely be explained by the toxicity of the material, administered in too high concentrations. Recently the importance of proper dosage was again emphasized by Schneider (156). In an evaluation of the effects of vertebrate endocrines on such functions as development and growth, fertility, metabolism, or regeneration, the normal physiological behavior of the tested invertebrates and its variability is not always sufficiently taken into consideration. Thus undoubtedly many of the reactions reported are unspecific, particularly if they are of an indefinite nature and if they are similarly observed after the administration of different endocrines. At present only those cases in which the responses are comparable with those known in the donors seem to indicate specific actions of vertebrate hormones on invertebrates and vice versa.

By way of final summary, the study of endocrine organs and their

functions is no longer restricted to vertebrates. Whereas the question of an active rôle of sex hormones in invertebrates is still controversial and the true hormonal nature of gene-controlled substances has yet to be confirmed, the rôle of endocrines in color change of arthropods and in development of insects is well established. The physico-chemical properties of invertebrate hormonal substances as well as their physiological actions are comparable with those of corresponding vertebrate materials. This is demonstrated by the fact that vertebrate and invertebrate endocrines may replace each other in certain actions. It should be kept in mind, however, that such physiological relations do not indicate anatomical homology of any one of the endocrine glands of invertebrates with those of vertebrates.

REFERENCES

(1) Abramowitz, A. A. Proc. Nat. Acad. Sci. **21**: 677, 1935.
(2) Abramowitz, A. A. Proc. Nat. Acad. Sci. **22**: 521, 1936.
(3) Abramowitz, A. A. Anat. Rec. **67**: 108, 1936–37.
(4) Abramowitz, A. A. Biol. Bull. **72**: 344, 1937.
(5) Abramowitz, A. A. J. exper. Zool. **76**: 407, 1937.
(6) Abramowitz, A. A. Physiol. Zool. **11**: 299, 1938.
(7) Abramowitz, A. A. J. Biol. Chem. **132**: 501, 1940.
(8) Abramowitz, A. A. and R. K. Abramowitz. Biol. Bull. **74**: 278, 1938.
(9) Abramowitz, R. K. and A. A. Abramowitz. Biol. Bull. **78**: 179, 1940.
(10) Arcangeli, A. Arch. zool. ital. **17**: 165, 1932.
(11) Atzler, M. Ztschr. vergl. Physiol. **13**: 505, 1930.
(12) Bacq, Z. M. and M. Florkin. Arch. intern. Physiol. **40**: 422, 1935.
(13) Bain, W. A. Quart. J. exper. Physiol. **19**: 297, 1929.
(14) Bauer, E. E. Arch. exper. Path. und Pharmakol. **163**: 602, 1932.
(15) Beauvallet, M. and C. Veil. C. R. Soc. Biol. **117**: 688, 1934.
(16) Becker, E. and E. Plagge. Biol. Zentralbl. **59**: 326, 1939.
(17) Benazzi, M. Boll. Zool. **10**: 99, 1939.
(18) Bodenstein, D. Ergebn. d. Biol. **13**: 174, 1936.
(19) Bodenstein, D. Arch. Entw. Mech. **137**: 474, 1938.
(20) Bodenstein, D. J. exper. Zool. **82**: 1, 1939.
(21) Bodenstein, D. J. exper. Zool. **82**: 329, 1939.
(22) Bodenstein, D. J. exper. Zool. **84**: 23, 1940.
(23) Bounhiol, J. J. C. R. Acad. Sc. **206**: 773, 1938.
(24) Bounhiol, J. J. Arch. zool. expér. gén. **81**: 54, 1939.
(25) Brecher, L. Arch. Entw. Mech. **102**: 501, 1924.
(26) Brecher, L. Arch. Entw. Mech. **102**: 517, 1924.
(27) Brinkmann, A. Bergens Mus. Skrifter nr. 18, 1–111, 1936.
(28) Brown, F. A., Jr. Proc. Nat. Acad. Sci. **19**: 327, 1933.
(29) Brown, F. A., Jr. J. Morph. **57**: 317, 1935.
(30) Brown, F. A., Jr. J. exper. Zool. **71**: 1, 1935.
(31) Brown, F. A., Jr. Proc. Nat. Acad. Sci. **24**: 551, 1938.

(32) Brown, F. A., Jr. Am. Nat. **73:** 247, 1939.
(33) Brown, F. A., Jr. Physiol. Zool. **13:** 343, 1940.
(34) Brown, F. A., Jr. and O. Cunningham. Biol. Bull. **77:** 104, 1939.
(35) Brown, F. A., Jr. and H. E. Ederstrom. Biol. Bull. **77:** 330, 1939.
(36) Brown, F. A., Jr. and A. Meglitsch. Biol. Bull. **79:** 409, 1940.
(37) Brown, F. A., Jr. and H. H. Scudamore. J. cell. and comp. Physiol. **15:** 103, 1940.
(38) Burge, W. E., G. C. Wickwire, A. M. Estes and L. D. Seager. Endocrinology **12:** 157, 1928.
(39) Burtt, E. T. Proc. Roy. Soc. London Ser. B. **124:** 13, 1937.
(40) Burtt, E. T. Proc. Roy. Soc. London Ser. B. **126:** 210, 1938.
(41) Butcher, E. O. J. exper. Zool. **57:** 1, 1930.
(42) Butenandt, A., W. Weidel and E. Becker. Naturwiss. **28:** 63, 1940.
(43) Callan, H. G. J. exper. Biol. **17:** 168, 1940.
(44) Carlson, S. P. Proc. Nat. Acad. Sci. **21:** 549, 1935.
(45) Carlson, S. P. Kgl. Fysiogr. Sällsk. Handl. **6:** 63, 1936.
(46) Ciabatti, O. and G. Floris. Scritti biol. **5:** 209, 1930.
(47) Clancy, E. B. Biol. Bull. **78:** 217, 1940.
(48) Craig, R. and W. M. Hoskins. Ann. Rev. Biochem. **9:** 617, 1940.
(49) Crozier, W. J. and E. Wolf. Biol. Bull. **77:** 126, 1939.
(50) Darby, H. H. Carnegie Inst. Washington Publ. Year book nr. **34:** 78, 1934–35.
(51) Day, M. F. Nature **145:** 264, 1940.
(52) De Bach, P. Ann. Ent. Soc. America **32:** 743, 1939.
(53) Dobzhansky, T. Arch. Entw. Mech. **123:** 719, 1931.
(54) Donahue, J. K. Endocrinol. **27:** 149, 1940.
(55) Dubois, R. C. R. Soc. Biol. **63:** 636, 1907.
(56) Edlén, A. Arch. Entw. Mech. **137:** 804, 1938.
(57) Elwyn, A. Biol. Bull. **75:** 370, 1938.
(58) Ephrussi, B. Am. Nat. **72:** 5, 1938.
(59) Fleischmann, W. Vergleichende Physiologie der inneren Sekretion. Wien, 1937.
(60) Fraenkel, G. Proc. Roy. Soc. London Ser. B. **118:** 1, 1935.
(61) Gaskell, J. F. Phil. Trans. Roy. Soc. London B. **205:** 153, 1914.
(62) Geigy, R. Rev. Suisse Zool. **38:** 187, 1931.
(63) Geigy, R. and W. Ochsé. Rev. Suisse Zool. **47:** 193, 1940.
(64) Giard, A. C. R. Acad. Sci. **103:** 84, 1886.
(65) Giersberg, H. Ztschr. vergl. Physiol. **7:** 657, 1928.
(66) Giersberg, H. Ztschr. vergl. Physiol. **9:** 523, 1929.
(67) Goldschmidt, R. Mechanismus und Physiologie der Geschlechtsbestimmung. Berlin, 1920.
(68) Goldschmidt, R. Die sexuellen Zwischenstufen. Monogr. Physiol. Pflanzen u. Tiere **23,** Berlin, 1931.
(69) Gottschweski, G. and J. Fischer. Naturwiss. **27:** 584, 1939.
(70) Grave, C. Carnegie Inst. Washington Publ. nr. **452:** 209, 1935.
(71) Hachlow, V. Arch. Entw. Mech. **125:** 26, 1932.
(72) Hadorn, E. Proc. Nat. Acad. Sci. **23:** 478, 1937.
(73) Hadorn, E. and J. Neel. Arch. Entw. Mech. **138:** 281, 1938.

(74) HAEMMERLI-BOVERI, V. Ztschr. vergl. Physiol. **4**: 668, 1926.
(75) HANKÓ, B. Arch. Entw. Mech. **34**: 477, 1912.
(76) HANSTRÖM, B. Proc. Nat. Acad. Sci. **21**: 584, 1935.
(77) HANSTRÖM, B. Kgl. Fysiogr. Sällsk. Handl. **6**: 58, 1936.
(78) HANSTRÖM, B. Kgl. Svensk. Vetensk. Handl. **16**: 3, 1937.
(79) HANSTRÖM, B. Lunds Univ. Årsskrift N.F. Avd. 2, **34**: nr. 16, pp. 1–17, 1938.
(80) HANSTRÖM, B. Hormones in invertebrates. Oxford, 1939.
(81) HARMS, W. Arch. Entw. Mech. **47**: 307, 1921.
(82) HERWERDEN, M. A. VAN Arch. mikr. Anat. u. Entw. Mech. **98**: 221, 1923.
(83) HERWERDEN, M. A. VAN Arch. mikr. Anat. u. Entw. Mech. **98**: 505, 1923.
(84) HEUMANN, A. Ztschr. wiss. Zool. **138**: 515, 1931.
(85) HEYL, H. L. Science **89**: 540, 1939.
(86) HUUS, J. Ascidiaceae. In: Handb. d. Zool., ed. by Kükenthal, W. and T. Krumbach, **5**, II: 545, 1937–40.
(87) IWANOFF, P. P. AND K. A. MESTSCHERSKAJA. Zool. Jahrb. Physiol. **55**: 281, 1935.
(88) JANDA, V. Mem. Soc. Sci. Bohème, Cl. Sci. p. 1–31, 1934.
(89) JANDA, V. Zool. Anz. **115**: 177, 1936.
(90) KALMUS, H. Ztschr. vergl. Physiol. **25**: 494, 1938.
(91) KALMUS, H. Ztschr. vergl. Physiol. **25**: 798, 1938.
(92) KALMUS, H. Ztschr. vergl. Physiol. **26**: 362, 1938–39.
(93) KALMUS, H. AND V. WALDES. Ztschr. vergl. Physiol. **23**: 712, 1936.
(94) KESTNER, O. Ztschr. vergl. Physiol. **15**: 159, 1931.
(95) KLEINHOLZ, L. H. Biol. Bull. **72**: 176, 1937.
(96) KLEINHOLZ, L. H. Biol. Bull. **75**: 510, 1938.
(97) KNOWLES, F. G. W. Pubbl. Staz. zool. Napoli **17**: 174, 1939.
(98) KOLLER, G. Verh. dtsch. zool. Ges. **30**: 128, 1925.
(99) KOLLER, G. Ztschr. vergl. Physiol. **5**: 191, 1927.
(100) KOLLER, G. Ztschr. vergl. Physiol. **8**: 601, 1928.
(101) KOLLER, G. Ztschr. vergl. Physiol. **12**: 632, 1930.
(102) KOLLER, G. Hormone bei wirbellosen Tieren. Leipzig, 1938.
(103) KOLLER, G. AND E. MEYER. Biol. Zentralbl. **50**: 759, 1930.
(104) KOPEĆ, S. Arch. Entw. Mech. **33**: 1, 1912.
(105) KOPEĆ, S. Biol. Bull. **42**: 323, 1922.
(106) KROPP, B. AND E. B. PERKINS. Biol. Bull. **64**: 28, 1933.
(107) KROPP, B. AND E. B. PERKINS. Biol. Bull. **64**: 226, 1933.
(108) KRUTA V. C. R. Soc. Biol. **119**: 397, 1935.
(109) KÜHN, A., E. CASPARI AND E. PLAGGE. Ges. Wiss. Göttingen, Nachr. a. d. Biol. **2**: 1, 1935.
(110) KÜHN, A. AND H. PIEPHO. Ges. Wiss. Göttingen, Nachr. a. d. Biol. **2**: 141, 1936.
(111) KÜHN, A. AND H. PIEPHO. Biol. Zbl. **58**: 12, 1938.
(112) LAW, L. W. Proc. Soc. Exper. Biol. and Med. **40**: 442, 1939.
(113) LEDRUT, J. AND G. UNGAR. Arch. intern. Physiol. **44**: 205, 1936–37.
(114) LE ROUX, M. L. Bull. Biol. France et Belg., suppl. **16**: 1, 1933.
(115) LINKE, O. Zool. Anz., Suppl. **7**: 164, 1934.
(116) LOEWE, S. Naturwiss. **19**: 775, 1931.

(117) LOEWE, S., W. RAUDENBUSCH, H. E. VOSS AND J. W. C. VAN HEURN. Biochem. Ztschr. **244:** 347, 1932.
(118) MARTINI, E. AND J. ACHUNDOW. Zool. Anz. **81:** 25, 1929.
(119) MAUSER, F. Biol. gen. (Vienna) **14:** 179, 1938.
(120) MEDVÉDEV, N. Med. Ž. vseukraïn. Akad. Nauk. **5:** 173, 1935.
(121) MEISENHEIMER, J. Zool. Anz. **32:** 393, 1908.
(122) MEISENHEIMER, J. Geschlecht und Geschlechter im Tierreiche. vol. 2, Jena, 1930.
(123) MELLANBY, K. Parasitol. **30:** 392, 1938.
(124) MELLO, F. DE Biol. Zentralbl. **60:** 174, 1940.
(125) MEYER, E. Zool. Jahrb. allg. Zool. u. Physiol. **49:** 231, 1931.
(126) MITOLO, M. Arch. di Sci. Biol. **24:** 33, 1938.
(127) MORI, Y. Ztschr. wiss. Zool. **144:** 289, 1933.
(128) MORI, Y. Ztschr. wiss. Zool. **144:** 573, 1933.
(129) NAVEZ, A. E. AND B. KROPP. Biol. Bull. **67:** 250, 1934.
(130) PAIK, S. M. Jap. J. med. Sci. Trans. IV, Pharm. **8:** 131, 1935.
(131) PARHON, C. J. AND H. DEREVICI. C. R. Soc. Biol. **110:** 643, 1932.
(132) PARKER, G. H. Proc. Am. Acad. Arts. Sci. **73:** 165, 1940.
(133) PERKINS, E. B. J. exper. Zool. **50:** 71, 1928.
(134) PERKINS, E. B. AND B. KROPP. Biol. Bull. **63:** 108, 1932.
(135) PERKINS, E. B. AND T. SNOOK. Proc. Nat. Acad. Sci. **17:** 282, 1931.
(136) PFEIFFER, I. W. J. exper. Zool. **82:** 439, 1939.
(137) PFLUGFELDER, O. Ztschr. wiss. Zool. **149:** 477, 1937.
(138) PFLUGFELDER, O. Ztschr. wiss. Zool. **150:** 451, 1938.
(139) PFLUGFELDER, O. Ztschr. wiss. Zool. **151:** 149, 1938.
(140) PFLUGFELDER, O. Ztschr. wiss. Zool. **152:** 159, 1939.
(141) PFLUGFELDER, O. Ztschr. wiss. Zool. **152:** 384, 1939.
(142) PIEPHO, H. Biol. Zentralbl. **58:** 356, 1938.
(143) PIEPHO, H. Biol. Zentralbl. **58:** 481, 1938.
(144) PIEPHO, H. Naturwiss. **27:** 675, 1939.
(145) PIEPHO, H. Biol. Zentralbl. **59:** 314, 1939.
(146) PLAGGE, E. Biol. Zentralbl. **58:** 1, 1938.
(147) PLAGGE, E. Ergebn. Biol. **17:** 105, 1939.
(148) PRELL, H. Zool. Jahrb. allg. Zool u. Physiol. **35:** 183, 1915.
(149) PRIEBATSCH, J. Ztschr. vergl. Physiol. **19:** 453, 1933.
(150) RANZI, S. Naturwiss. **27:** 660, 1939.
(151) REMPEL, J. G. J. exper. Zool. **84:** 261, 1940.
(152) ROAF, H. E. Quart. J. Exper. Physiol. **4:** 89, 1911.
(153) SCHARRER, B. J. comp. Neurol. **74:** 93, 1941.
(154) SCHARRER, B. AND E. HADORN. Proc. Nat. Acad. Sci. **24:** 236, 1938.
(155) SCHARRER, E. AND B. SCHARRER. Res. Publ. Ass. nerv. ment. Dis. **20:** 170, 1940.
(156) SCHNEIDER, B. A. Quart. Rev. Biol. **14:** 289, 1939.
(157) SCHNEIDER, B. A. J. exper. Zool. **84:** 113, 1940.
(158) SCHNEIDER, B. A. J. exper. Zool. **84:** 141, 1940.
(159) SCHNEIDER, B. A. J. exper. Zool. **84:** 163, 1940.
(160) SCHNEIDER, B. A. J. exper. Zool. **84:** 179, 1940.
(161) SCHNEIDER, B. A. J. exper. Zool. **84:** 189, 1940.

(162) SCHWERDTFEGER, H. Arch. exper. Path. und Pharmakol. **163:** 487, 1932.
(163) SERENI, E. Am. J. Physiol. **90:** 512, 1929.
(164) SERENI, E. Boll. Soc. Ital. Biol. sperim. **4:** 749, 1929.
(165) SHIINO, S. M. Mem. Coll. Sci. Kyoto, B. **7:** 63, 1931–32.
(166) SMITH, G. Quart. J. micr. Sci. **57:** 251, 1912.
(167) SMITH, H. G. Proc. Roy. Soc. London B. **125:** 250, 1938.
(168) SPAUL, E. A. J. exper. Biol. **5:** 212, 1928.
(169) STÅHL, F. Lunds Univ. Årsskr., N. F. 2, **34:** nr. 12, pp. 1–20, 1938.
(170) STEIDLE, H. Arch. exper. Path. und Pharmakol. **157:** 89, 1930.
(171) STEPHENSON, E. M. Nature **133:** 912, 1934.
(172) STURTEVANT, A. H. Proc. Soc. exper. Biol. Med. **17:** 70, 1920.
(173) TATUM, E. L. Proc. Nat. Acad. Sci. **25:** 486, 1939.
(174) TATUM, E. L. AND G. W. BEADLE. Biol. Bull. **77:** 415, 1939.
(175) THOMSEN, E. Nature **145:** 28, 1940.
(176) TOWNSEND, G. F. AND C. C. LUCAS. Science **92:** 43, 1940.
(177) TUCKER, B. W. Quart. J. micr. Sci. **74:** 1, 1931.
(178) TURNER, C. L. Biol. Bull. **69:** 269, 1935.
(179) VANDEL, A. C. R. Acad. Sci. **170:** 249, 1920.
(180) VANDEL, A. Bull. Biol. France et Belg. **64:** 457, 1930.
(181) WEISS, P. Biol. Zentralbl. **48:** 69, 1928.
(182) WELLS, G. P. J. exper. Biol. **14:** 117, 1937.
(183) WELSH, J. H. Proc. Nat. Acad. Sci. **23:** 458, 1937.
(184) WELSH, J. H. Quart. Rev. Biol. **13:** 123, 1938.
(185) WELSH, J. H. Biol. Bull. **77:** 119, 1939.
(186) WENSE, T. Arch. exper. Path. und Pharmakol. **179:** 475, 1935.
(187) WENSE, T. Wirkungen und Vorkommen von Hormonen bei wirbellosen Tieren. Leipzig, 1938.
(188) WENSE, T. Pflüger's Arch. **241:** 284, 1939.
(189) WHEELER, W. M. J. exper. Zool. **8:** 377, 1910.
(190) WHEELER, W. M. J. exper. Zool. **50:** 165, 1928.
(191) WHITING, P. W. AND A. R. WHITING. J. Genet. **29:** 311, 1934.
(192) WIGGLESWORTH, V. B. Quart. J. micr. Sci. **77:** 191, 1934.
(193) WIGGLESWORTH, V. B. Quart. J. micr. Sci. **79:** 91, 1936.
(194) WIGGLESWORTH, V. B. Naturwiss. **27:** 301, 1939.
(195) WIGGLESWORTH, V. B. J. exper. Biol. **17:** 201, 1940.

ADDENDA.[5]

(196) BODENSTEIN, D. J. exper. Zool. **86:** 87, 1941.
(197) BROWN, F. A., JR. AND O. CUNNINGHAM. Biol. Bull. **81,** 1941. In press.
(198) BROWN, F. A., JR. AND H. E. EDERSTROM. J. exper. Zool. **85:** 53, 1940.
(199) BROWN, F. A., JR. AND V. J. WULFF. Anat. Rec. **78** suppl.: 139, 1940.
(200) DAY, M. F. Anat. Rec. **78** suppl.: 150, 1940.
(201) DAY, M. F. Ann. Ent. Soc. America, in press.
(202) HESS, W. N. AND R. L. BACON. Anat. Rec. **78** suppl.: 150, 1940.

[5] References 196 ff. were added after this article had been completed, October 1, 1940, when publication was postponed.

(203) KLEINHOLZ, L. H. Anat. Rec. **78** suppl.: 70, 1940.
(204) PFEIFFER, I. W. Anat. Rec. **78** suppl.: 39, 1940.
(205) SCHARRER, B. Biol. Bull. **81,** 1941. In press.
(206) SMITH, R. I. Biol. Bull. **79:** 145, 1940.
(207) VOGT, M. Biol. Zentralbl. **60:** 479, 1940.
(208) WELSH, J. H. J. exper. Zool. **86:** 35, 1941.
(209) WILLIAMS, C. M. Anat. Rec. **78** suppl.: 99, 1940.

ORGANIC ACID-SOLUBLE PHOSPHORUS COMPOUNDS OF THE BLOOD

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This review is concerned mainly with the functional significance of the organic acid-soluble phosphorus compounds of blood. The inorganic phosphorus of the blood also is dealt with insofar as problems concerning the inorganic and organic compounds are closely interrelated. More complete discussion of the chemistry and general metabolism of these and related compounds in blood, muscle and other tissues may be found in the reviews of Kay and Byrom (74), Peters and Van Slyke (105), Kay (73a, b,) Schmidt and Greenberg (122), Robison (114, 115), Nissen (103), Lohman (88), Baumann and Stare (14). Recent investigations in which radioactive phosphorus has been used for the study of these compounds have been reviewed by Greenberg (44) and by Hevesy (60).

Those reviews cite evidence aptly described by Kay (73a) as "sometimes sound, sometimes not quite so sound," that the following acid-soluble phosphorus compounds are present or may be formed in blood: inorganic phosphates, hexose mono- and diphosphates, triosephosphates, adenylic acid, adenosinetriphosphate, phosphopyruvic acid, diphosphoglyceric acid, diphospho-α-ketotrihydroxyadipic acid, cozymase, (diphosphopyridine nucleotide), triphosphopyridine nucleotide, and cocarboxylase. To this list was added recently phytic acid, present in avian blood corpuscles (Rapoport, 108).

Studies of the organic phosphorus compounds in blood in different physiologic and pathologic conditions have been limited necessarily to those substances for which convenient analytical procedures have been devised. Limitations of methods account for the fact that existing knowledge of variations in the distribution of acid-soluble P compounds in the blood in disease is practically limited to the inorganic and total acid-soluble P, adenosinetriphosphate, and diphosphoglycerate. Methods in current use are cited here only briefly, since they have been discussed extensively in the above mentioned reviews.

METHODS. The separation of phosphorus compounds of the blood according to their solubility in acid protein precipitants such as trichloroacetic and picric acids apparently was first made by Greenwald (45). The method which appears to be the most used in current investigations for the determination of inorganic P and total P in the deproteinized filtrates of blood is that of Fiske and Subbarow (39), but a host of other methods may be found cited in the reviews listed in the first paragraph of this paper.

Various methods have been devised for the fractionation of the organic phosphates. Kay and Robison (75) distinguished two fractions of the organic acid-soluble P, respectively hydrolyzable and non-hydrolyzable by bone phosphatase. Another method of fractionation, introduced by Lohmann (86) and first applied to blood analysis by Bomskov (15), was based on differences in the rate of hydrolysis of various phosphoric esters by mineral acids. Several fractions can be determined more or less accurately by this procedure, but knowledge of the nature of the esters thus determined is a prerequisite. The fraction most easily split is the so-called pyrophosphate, liberated by 7 minutes' boiling with N HCl or H_2SO_4; this represents two-thirds of the adenosinetriphosphate P. If such acid hydrolysis be continued 150 minutes, other phosphoric esters, including a large part of the adenylic acid, are decomposed. Approximately two-thirds of the organic acid-soluble P, remaining unhydrolyzed after 150 minutes, may require 48 hours for complete hydrolysis; this is usually designated the non-hydrolyzable fraction, and is composed of diphosphoglycerate and other compounds. There is of course some overlapping between these fractions.

Fractionation of the phosphoric esters by enzyme and acid hydrolysis appear to lead to fairly similar results. Kerr and Antaki (78) determined the breakdown of organic phosphates in several species of blood by bone and kidney phosphatases and by acid hydrolysis. Their results indicated that adenosinetriphosphate appeared to be the principal substrate of phosphatase, although in pig and dog bloods an unidentified compound contributed significantly to the phosphate liberated by the enzyme. Warweg and Stearns (134) determined the velocity constants for enzyme and acid hydrolysis of the organic phosphates in human blood filtrates and of pure phosphoric esters, with results indicating that the so-called non-hydrolyzable fraction of the organic acid-soluble P corresponded with pure diphosphoglycerate isolated from pig blood.

Another method for the fractionation of the organic acid-soluble P compounds of blood is based on the different solubilities of the barium salts of these compounds (Eggleton and Eggleton, 26). The barium salts of phosphoric acid, of adenosinetriphosphate, and of phosphoglyceric acid are insoluble in water, whereas creatine phosphate, hexosemonophosphate, adenylic acid and other phosphoric esters yield soluble barium salts. The primary separation by barium precipitation is usually followed by further fractionation by means of acid-hydrolysis, or by determination of the reducing power of the precipitate. A method for the determination of hexosemonophosphate, based on the solubility of the barium salts, and the reducing power and phosphorus content of the precipitate, was devised by Cori and Cori (23) and applied to blood analysis by Morgulis and Munsell (100). The identity of the compound thus determined in blood, however, is not definitely established.

Methods for the determination of nucleotides in blood have been developed by Buell (18) and by Kerr and Blish (79). Kerr and Daoud (80) and Kerr (77) reported data on the blood cells of 24 species indicating that in nearly all of these bloods adeninenucleotide and readily hydrolyzable P were present in the proportion to be expected if they were combined as adenosinetriphosphate. Fiske (38) reported direct evidence that all the adenylic acid of rabbit blood was present as adenosinetriphosphate.

A quantitative method for the determination of the phosphoric esters of glyceric acid was devised by Rapoport (106), based on a color reaction given by glyceric acid and its esters when treated with naphthoresorcinol in concentrated sulfuric acid.

For the determination of phytic acid, the method of Michel-Durand (99) has been modified and adapted to blood analysis by Leva and Rapoport (unpublished).

Distribution in plasma and cells. The organic acid-soluble P compounds in blood are practically confined to the cells. The small amount found in the plasma (usually less than 0.5 mgm. per 100 cc.) generally has been regarded as negligible although Robison (113) and Martland and Robison (94) suggested that it might have functional significance. Analysis of whole blood has been adopted by most investigators because common experience taught that changes might occur in cells during their separation from plasma by centrifugation, with more or less rapid breakdown of the more labile compounds and liberation of inorganic P (Kerr and Daoud, 80). In this case the cellular concentrations of the organic phosphates are calculated from whole blood values, by means

of the per cent cell volume determined by a suitable hematocrit method, and the organic acid-soluble P in the plasma usually is not taken into account. Constituents of the white cells are of course included in analyses of whole blood.

The amount of inorganic P normally present in blood cells has been the subject of considerable debate and conflicting opinions (Peters and Van Slyke, 105; Halpern, 57; Nissen, 103; Freudenberg, 41). Normally the concentration of inorganic P in the cells is considerably less than in the plasma, but its distribution does not correspond with the distribution of other diffusible ions such as H^+, Cl^-, and HCO_3^-, between the cells and serum. The concentration of inorganic P in the blood cells is probably governed mainly by factors which affect the rates of synthesis and decomposition of the phosphoric esters, rather than factors which are believed to govern the Donnan equilibrium (127). Thus the concentration of inorganic P in the cells in different conditions has been found to vary between 0 and values considerably higher than those found in the corresponding plasma.

The question of the passage of phosphorus between cells and plasma has been somewhat clarified by recent studies in which "labelled" radioactive phosphorus (P^{32}) has been used to trace the turnover of phosphorus in the blood. Hevesy and Aten (61) studied the rate at which labelled phosphate ions added to the plasma of rabbit's blood penetrated the corpuscles, and obtained evidence suggesting that "the penetration of the labelled inorganic phosphate into the corpuscles is a comparatively slow process while the process in which the labelled inorganic phosphate is incorporated into the easily hydrolysable organic compounds inside the corpuscles is a fast one." Eisenman and co-workers (27) also used radioactive isotopes to study the permeability of erythrocytes *in vitro* to potassium, sodium, and phosphate ions. They found evidence of active entry of the labelled P^{32} into the blood cells and of its synthesis into organic phosphates, and of inhibition of this process at low temperatures. Inhibition of the transfer of inorganic P between cells and plasma by low temperatures was also discussed by others (57, 127). Eisenman and co-workers (27) concluded from their experiments that the transfer of phosphate across the cell membrane was associated with enzymatic processes rather than with diffusion. It should be noted, however, that diffusion of phosphate is not excluded by such findings, and that the diffusion gradient might be affected by rates of synthesis and decomposition of phosphoric esters within the cells.

Species. Studies of the distribution of phosphorus compounds and of phosphatases in the bloods of different species of animals have yielded data which are of considerable interest in connection with evolutionary relationships among the species, as well as pointing to possible differences and similarities in the phosphorus metabolism of the various species.

Kay (70) reported studies of the distribution of acid-soluble P in the bloods of 11 mammalian species. His data on the concentrations of organic acid-soluble P in the blood cells correspond with data similarly determined later by other investigators, and his values for the fraction of the ester P hydrolyzed by bone enzyme correspond roughly with the concentrations of adenosinetriphosphate found by others in the bloods of those 11 species. Kerr and Daoud (80) and Kerr (77) determined the concentrations of inorganic P, adenosinetriphosphate, and organic acid-soluble P in the bloods of 28 species of vertebrates; and McCay (95), the inorganic P and organic acid-soluble P in the bloods of 3 other lower vertebrates.

Recently we reported (112) data on the distribution of inorganic P, organic acid-soluble P, adenosinetriphosphate, diphosphoglycerate, and phytic acid P in the bloods of 46 vertebrates: 22 mammalian, 12 avian, 6 reptilian, 4 amphibian, and 2 species of fish. In most of the mammalian bloods the concentration of organic acid-soluble P in the cells was between 50 and 100 mgm. per 100 cc., with a high proportion (usually one-half) of phosphoglycerate. In a small group of ungulates (beef, sheep, goats, deer) the concentration of organic acid-soluble P in the cells was much lower, 9 to 15 mgm. per 100 cc., with only traces of phosphorylated glyceric acid. (See discussion of age differences, p. 415.) In all bird bloods the concentration of organic acid-soluble P in the cells was high, 90 to 135 mgm. per 100 cc., with a large proportion of phytic acid P, 49 to 87 mgm. per 100 cc. The distribution of phosphorus in the bloods of the avian species appeared to be more uniform than that found among different species of other classes studied. The avian species also are known to show relatively few differences in anatomic structure when compared with the structural diversity found among the members of other classes of animals. The data on 6 species of reptiles indicated a great diversity in the distribution of phosphorus in the bloods of this class. In snake blood cells the concentration of organic acid-soluble P was high with a large proportion of adenosinetriphosphate. In alligator blood cells the concentration of organic acid-soluble P was low. Turtle blood alone among all species other

than avian contained phytic acid. Turtle blood also was found to have a distribution of phosphatase and phytase similar to that found in bird bloods (unpublished work). Such findings suggest a very old phylogenetic origin of phytic acid, probably among the primitive reptiles. Phosphoglycerate was not found in the bloods of any species which normally have nucleated erythrocytes. Adenosinetriphosphate was found in widely varying concentrations in the blood cells of all species.

Sex. There appears to be no significant difference in the distribution of acid-soluble P between the bloods of males and females of any species. Kay and Byrom (74) found practically identical concentrations of organic phosphates in the blood cells of normal men and women.

Age. Although the level of inorganic P in the blood is generally recognized to be higher in young animals than in older ones, only a few figures are available on changes in the organic acid-soluble P compounds of the blood at different age periods in different species of animals.

Young rats have considerably higher concentrations of inorganic P, organic acid-soluble P, adenosinetriphosphate, and diphosphoglycerate in the blood cells than older rats. These concentrations fall progressively until adult levels are reached around the sixth week of life (Kay, 72; Rapoport and Guest, 109).

Green and Macaskall (43) and Malan (91) have reported high concentrations of organic acid-soluble P and of potassium in the bloods of lambs and calves, compared with concentrations found in the bloods of grown sheep and beef. We also have found relatively high concentrations (around 60 mgm. per 100 cc.) of organic acid-soluble P in the blood cells of calves during the first week of life, with half of this accounted for in the phosphoglycerate fraction (unpublished work). This was in contrast with the cells of adult beef blood which have a low concentration of organic acid-soluble P with only a trace of phosphoglycerate. Such data might indicate that the blood cells in the young of all mammalian species have similar high concentrations of phosphorus and potassium, and that the great differences found between the blood cells of adult individuals of various mammalian species are due to secondary developments of ontogeny.

Anderson and Elvehjem (3) reported lower levels of total acid-soluble P in the whole blood of mature dogs than of young dogs, but no values for the volume of cells in these bloods were included in the report. Nissen (103) reported extensive studies of the distribution of phosphorus in the whole blood of several species, with regard to age, but gave no data on the volume of cells in the blood.

Stearns and Warweg (128, 129) found the average values for ester P in the blood cells of infants to increase somewhat from birth through the first year of life and thereafter to decrease gradually in the succeeding years of childhood.

Types of cells. Except for a few studies of reticulocytes, little attention appears to have been paid to the distribution of acid-soluble P in blood cells other than mature erythrocytes. Data concerning human reticulocytes have been reported by Kay (71) who studied the distribution of phosphorus in the bloods of patients with splenic anemia, acholuric jaundice, hemolytic icterus during crises, and pernicious anemia following treatment with liver extract. In such bloods the concentration of organic acid-soluble P in the cells was greatly increased, to as high as 90 mgm. per 100 cc. There was indirect evidence also that nucleoprotein, generally believed to be absent in mature erythrocytes, was present in these bloods in amounts roughly proportional to the reticulocyte count.

We have determined the distribution of acid-soluble P in rabbit blood in which practically 100 per cent of the erythrocytes showed reticulation following repeated injections of the rabbits with phenylhydrazine (unpublished studies). The concentration of organic acid-soluble P in the cells of such blood was high, and the increase was largely accounted for in the adenosinetriphosphate fraction which rose from initial levels of between 10 and 14 mgm. per 100 cc. to above 30 mgm. per 100 cc. The high concentrations of adenosinetriphosphate in the cells appeared to be correlated with high values for mean volume, mean diameter, and amount of reticulation—characteristics of immature cells. While the origin of the increased amounts of adenosinetriphosphate is unknown it appears likely that adenylic acid may be derived from nucleic acid of the disintegrating nucleus.

Henriques and Ørskov (59) found an increased concentration of magnesium and potassium in the blood cells of rabbits and dogs with high reticulocytosis following injections of phenylhydrazine. We confirmed these findings in the unpublished experiments referred to in the preceding paragraph. In the reticulocytes a high concentration of adenosinetriphosphate and of magnesium possibly may be linked with certain known characteristics of the metabolism of these cells; namely, a high glycolytic rate compared with that of mature erythrocytes and a vigorous aerobic metabolism that is absent in mature erythrocytes (131, 34). Adenosinetriphosphate participates in both aerobic and anaerobic metabolism of blood and tissue cells, and magnesium has

been shown to be essential to the functioning of adenosinetriphosphate as a coenzyme (see review by Kay, 73). Also, the porphyrin found to be present by Watson (135) may be concerned with the aerobic metabolism and thus linked indirectly with the metabolism of adenosinetriphosphate in the reticulocytes. The finding of increased concentrations of potassium, magnesium, and phosphorus in reticulocytes suggests a fundamental similarity among immature blood cells of various mammalian species, in contrast with the great differences in composition of mature blood cells of these species.

Byrom and Kay (21) reported a few observations on the distribution of acid-soluble P in the leucocytes of the bloods of 3 normal persons, and of patients suffering with leukemia. The concentration of organic acid-soluble P in the leucocytes of these bloods ranged from 59 to 84 mgm. per 100 cc., with a high proportion of enzyme hydrolyzable P. We have found no other studies of organic acid-soluble P in leucocytes reported in recent literature.

Glycolysis. The early literature on relationships between phosphorus compounds of the blood and glycolysis is reviewed in articles cited in the first paragraph of this paper, and by Lundsgaard (89), Roche and Roche (118) and Guest (46). The early studies yielded evidence that the maintenance of a normal level of adenosinetriphosphate and other phosphoric esters in the blood was due to their continued synthesis and decomposition during glycolysis (Barrenscheen and co-workers, 10, 11, 12; Meyerhof, 97; Engelhardt and co-workers, 29, 31, 33; Roche and Roche, 118). A new approach to the problems of blood glycolysis was afforded by the work of Embden, Meyerhof, and Parnas who formulated a theory concerning the dismutations of phosphorus compounds connected with anaerobic breakdown of carbohydrate in muscle and yeast (Meyerhof, 98; Needham, 101; Parnas, 104). Roche and Roche (119) found the Harden-Young ester (fructose diphosphate) but not glucose to be metabolized by blood hemolysates. Dische showed that glucose was phosphorylated in blood only through the agency of adenosinetriphosphate and that fructose diphosphate and triosephosphate were intermediary products formed during glycolysis. Dische was the first to suggest the essential rôle of pyruvic acid in the glycolytic process and the coupling of the oxidation-reduction reactions through which phosphoglyceric acid is formed with esterification of inorganic P and adenylic acid. He stressed the apparent self-regulatory character of the reactions comprising the glycolytic cycle in blood (24). Several investigators (Barrenscheen and Beneschovsky, 9; Schuchardt and

Vercellone, 123; Braunstein, 16) found that monophosphoglycerate was readily attacked by blood hemolysates with the liberation of pyruvic acid and formation of phosphopyruvic acid; but that diphosphoglycerate was not decomposed by blood hemolysates. Later, however, diphosphoglycerate was found to be decomposed by intact blood cells *in vitro* (110).

Elsewhere (51) we have presented a simplified diagram plausibly representing the principal steps of the glycolytic cycle in blood, as suggested by the theories of Embden, Meyerhof, Parnas, Dische and others.

In forming diphosphoglycerate as an intermediary product of glycolysis the blood cells differ from cells of other tissues where monophosphoglyceric acid is known to be formed in the anaerobic breakdown of carbohydrates. Moreover, little is known concerning the intermediate steps in the synthesis and decomposition of diphosphoglycerate in the blood cells, compared with present knowledge of the reactions involving monophosphoglyceric acid. Jost in 1927 (68) suggested that diphosphoglycerate probably played an important rôle in blood glycolysis, but more definite evidence of this than he presented was lacking until recently. Using radioactive phosphorus, Hevesy and co-workers (61, 62) found that the diphosphoglycerate normally present in blood was constantly renewed at a rapid rate *in vivo* and *in vitro*. In unpublished studies of 10 species of blood, we have found that when blood cells were incubated in the presence of NaF and pyruvate the concentration of phosphoglycerate increased in all as expected if the breakdown of glucose occurred according to the Embden-Meyerhof theory. The phosphoglycerate thus accumulated in human and rabbit blood was found to be the diphosphoric ester. However, the phosphoglycerate thus formed in beef blood (which normally contains no phosphoglycerate) proved to be the monophosphoric ester, indicating that monophosphoglyceric acid may be the physiologic intermediate in the glycolytic process of this blood. Recent studies reported from Warburg's laboratory may lead to a better understanding of the mechanism of formation of diphosphoglycerate in blood cells and of relationships between the mono- and diphosphoric esters. Using purified enzyme preparations from yeast, Negelein and Broemel (102) found a labile compound formed as a precursor of monophosphoglyceric acid. This substance appeared to be a diphosphoglyceric acid but differed in its constitution from the stable 2, 3-diphosphoglyceric acid of blood cells. Monophosphoglycerate was formed from the labile ester by dephos-

phorylation. These findings suggest that in blood cells which normally have a high content of diphosphoglyceric acid a labile diphosphoglycerate may be transformed into the stable isomer, whereas in other cells a dephosphorylation of the labile compound with formation of monophosphoglyceric acid takes place.

In the breakdown of diphosphoglyceric acid in blood it has been suggested that rephosphorylation of adenylic acid takes place at the expense of diphosphoglycerate (110), but details of this mechanism are unknown. Whether monophosphoglycerate or phosphopyruvate or both are intermediary products of the degradation is uncertain.

There is evidence that an unidentified phosphoric ester, comprising about 5 to 10 per cent of the organic acid-soluble P in human and rabbit blood, also takes part in glycolysis. This substance is characterized by a water soluble barium salt and is probably a carbohydrate ester. Preliminary experiments with radioactive phosphorus indicate that this fraction is rapidly rejuvenated, and other findings suggest that it may be a precursor of phosphoglyceric acid.

In nucleated erythrocytes, organic acid-soluble P compounds take part in both aerobic and anaerobic processes. Several investigators reported evidence indicating that adenosinetriphosphate in avian blood participated both in respiration and in glycolysis, but that it was maintained at its normal level only during aerobic conditions (6, 29, 30). Lyubimova (85) reported similar findings on the adenosinetriphosphate in rabbit leucocytes. Studies of Braunstein and Severin (17, 124) on avian erythrocytes suggest that adenosinetriphosphate also may be concerned with the metabolism of protein. Although avian blood normally contains no phosphoglycerate, we have found that phosphoglycerate accumulated in avian blood cells during incubation in the presence of NaF and pyruvate (unpublished work). This finding again lends further support to the validity of the Embden-Meyerhof theory of glycolysis. Phytic acid, present in large amounts in bird and turtle blood cells, appears not to take part in the glycolytic process, but undergoes a slow aerobic turnover.

Differences in concentration and distribution of phosphorus compounds in the bloods of different species may depend on either qualitative or quantitative differences in any of the numerous steps in the glycolytic process as it occurs in different bloods; also, differences in glycolytic rates may be related to differences in the normal concentration and distribution of the acid-soluble P compounds in the different bloods. Erythrocytes which are normally characterized by a low con-

centration of organic acid-soluble P, as in the ungulates, generally show a low rate of glycolysis. However, other species of erythrocytes characterized by higher concentrations of organic acid-soluble P show widely varying rates of glycolysis: human and rabbit bloods show rapid glycolysis, but pig blood with a much higher concentration of organic acid-soluble P in the erythrocytes has a low glycolytic rate. Barrenscheen and Vasarhelyi (13) suggested a rough correlation between glycolytic rate and the concentration of adenosinetriphosphate in blood cells of different species (for comments, see Kerr and Daoud, 80). Engelhardt and Ljubimowa (33) found that the rate of glycolysis in bloods of different species was correlated with the amount of organic acid-soluble P that was broken down when the blood was incubated in the absence of glucose. In addition to differences in the distribution of phosphorus compounds, however, the pH, ionic milieu, and concentrations of various coenzymes also must be considered as factors which influence the rate of glycolysis in different bloods and in different conditions. The permeability of erythrocytes to glucose has been shown by Kolotilova and Engelhardt (32, 83) to be another factor influencing the glycolytic rate in different bloods.

In experiments with blood *in vitro* Lawaczeck (84) and others (41, 93, 120) found that shifts in pH of the blood from normal toward greater acidity led to a rapid decrease in concentration of the organic acid-soluble P in the cells; this decrease was found later to be due mainly to decomposition of diphosphoglycerate (110). Roche and Roche (117) found that with increased acidity of blood the glycolytic rate diminished. Recent experiments with radioactive P (unpublished work) indicate that the rate of turnover of phosphorus in the phosphoric esters is reduced with increased acidity of the blood. Changes of pH toward greater alkalinity than normal tend to produce effects opposite to those of increased acidity. The changes in pH may be assumed to concern mainly the processes preceding the oxidation-reduction reactions which lead to the formation of phosphoglycerate and lactic acid, most likely the phosphorylation of glucose by adenosinetriphosphate. The decomposition of diphosphoglycerate with rephosphorylation of adenylic acid seems to be less affected by changes of pH.

Numerous investigators who have studied the rate of glycolysis in human blood *in vitro* have found fairly constant rates in health and widely varying rates in disease (see reviews by John, 67; Falcon-Lesses, 36; Barer, 8). Data on the rates of glycolysis and concomitant changes of inorganic P in the bloods of infants and children with various diseases

were reported by Guest (47). Rapid glycolytic rates and rapid liberation of inorganic P were observed in blood samples from patients suffering with several different conditions: most notably, gastrointestinal intoxication, and nephritis with acidosis. Thus not only glycolysis but also the dismutations of organic phosphates in the blood may be affected by conditions arising in different diseases. Such alterations of the glycolytic process may be related to disturbances in the metabolism of other tissues.

Relation to general phosphorus metabolism. Since the inorganic P carried in the blood is subject to esterification and subsequent liberation through reactions of the glycolytic cycle, the phosphoric esters in the blood cells may be said to participate to some extent in the general turnover of phosphorus in the body. Observations on several conditions to be discussed later in this review suggest that the phosphoric esters in the blood cells serve as carriers of phosphorus which may be utilized in tissues or may leave the body in excreta. Present knowledge makes it difficult to estimate the relative importance of this phosphorus-carrying rôle of the phosphoric esters in the blood cells, or the extent to which these esters are actually concerned in the general phosphorus metabolism of the body. There is reason to believe that the turnover of phosphorus between plasma and cells is small compared with the total transport of inorganic P in the blood. It does appear, however, that the concentration of organic acid-soluble P in the blood cells constitutes an index of the *state* of the labile phosphorus reserves of the body, inasmuch as the concentrations of the phosphoric esters in the blood cells decrease or increase as those reserves are exhausted or replenished or are actively mobilized. These relationships are discussed in later sections of this paper dealing with acidosis, nephritis, rickets, etc.

Ionic equilibrium in the blood. In 1923 Van Slyke, Wu and McLean (130) found the buffer value for the non-diffusible constituents of the red blood cells of a horse much higher than should be expected from their hemoglobin content, and ascribed this difference to the conjugated phosphates. Similarly, Maizels and Paterson (90) found that a considerable part of the base-binding property of the erythrocytes of patients with hypochromic anemia was due to non-diffusible acids other than hemoglobin. Guest and Andrus (48) observed a striking reciprocal relationship between decreases of chloride and increases of organic acid-soluble P in the blood cells of dogs after pyloric obstruction. Ashley and Guest (5) reported similar relationships between changes of Cl and organic phosphates in the blood cells of dogs and rabbits

after abrupt suppression of renal function. Such observations indicated that the phosphoric esters not only comprised a considerable part of the anions in normal blood cells, but that they also played an important rôle in adjustments of the acid-base equilibrium of the blood in pathologic conditions.

Increased knowledge of the nature of the organic acid-soluble phosphates now makes possible a fairly complete summation of the electrolytes in blood cells. This has been demonstrated by analyses of dog blood, before and after pyloric obstruction, and of human blood during and after recovery from the severe acidosis of diabetic coma (51, 52, 111). The anion equivalencies of the organic phosphates were calculated by means of data on the titration curves of adenosinetriphosphate, determined by Lohmann (87), and of diphosphoglycerate, determined by Kiessling (81) and by Rubin (121). The anion equivalency of diphosphoglycerate in human and dog blood cells is normally around 30 m.eq. per kgm. of water. In severe acidosis its concentration was practically 0, and following pyloric obstruction its concentration was doubled. In the reported examples of data on each of these types of blood, the sums of the determined anions in the cells (Cl, HCO_3, hemoglobin, and the organic phosphates) agreed closely with the concentrations of total base determined in the cells—indicating that practically all the anions in the cells had been accounted for.

As non-diffusible anions the organic acid-soluble P compounds in the blood cells appear to influence the distribution of diffusible ions between serum and cells in the manner postulated by Van Slyke, Wu and McLean (130), conforming in a general way with Donnan's law concerning the distribution of diffusible and non-diffusible ions on two sides of a semi-permeable membrane. This was demonstrated (52, 111) by calculations based on the data referred to in the preceding paragraph. Using the formulae evolved by Van Slyke, Wu and McLean (130) and taking into consideration certain correction factors determined since the date of their studies, distribution ratios were calculated from data on the non-diffusible anions and compared with observed distribution ratios of the diffusible ions H^+, Cl^- and HCO_3^-. The agreement found among these ratios indicated that the distribution of the diffusible ions between the cells and serum depended mainly on the concentrations and anion equivalencies of hemoglobin and diphosphoglycerate, the two most important non-diffusible constituents of the cells.

In evaluating thus the rôle of diphosphoglycerate and hemoglobin as non-diffusible anions in the cells, it is to be emphasized that the im-

portant adjustments in anion equivalency of these two substances occur differently. In different conditions the anion equivalency of hemoglobin may change markedly with shifts in pH of the blood, but the hemoglobin content of the circulating cell does not change during short periods of acute acidosis or alkalosis. While the diphosphoglycerate also changes its anion equivalency somewhat with shifts in pH, its changes in concentration in such conditions are far more important. In this respect the diphosphoglycerate is actually more important than hemoglobin as a factor in the adjustments to changes in concentration of the diffusible electrolytes in the blood which take place in various pathologic conditions.

CHANGES IN THE DISTRIBUTION OF ACID-SOLUBLE PHOSPHORUS IN THE BLOOD IN DISEASE AND IN EXPERIMENTAL CONDITIONS. *Effects of acidosis.* In subjects with adequate renal function[1], the rapid development of acidosis due to many different causes is accompanied by a greatly increased excretion of phosphates in the urine. Excessive phosphaturia in human diabetes was observed around 1900 by Von Noorden, Mandel and Lusk and others and in 1907 Fitz, Alsberg and Henderson made the important observation that chloride acidosis experimentally produced in rabbits led to a greatly increased phosphaturia. Early work on this subject is reviewed by Forbes and Keith (40). Subsequently the fact that the excretion of phosphates serves to conserve base stores in the body in acidosis came to be well appreciated, but for several years little was learned concerning the nature of other adjustments which might be concerned in this physiologic mechanism. In 1924 Haldane, Wigglesworth and Kay (56) found that acidosis induced by the ingestion of ammonium chloride was associated with a greatly reduced concentration of organic acid-soluble P in the blood cells. Kay (69) found that this reduction was in the fraction not hydrolyzed by bone enzyme. In 1929 Byrom (19) found a similar reduction in concentration of organic acid-soluble P in the blood cells in diabetic acidosis, and also found these phosphoric esters in the blood cells to be restored to a normal concentration following effective insulin therapy. Similar findings in diabetic acidosis were reported by Meier and Thoenes (96). In 1937 Rapoport (107) identified as diphosphoglycerate the fraction of the organic acid-soluble P in the blood cells that decreased during ammonium chloride acidosis. This fraction

[1] Suppression of renal function from any cause, with failure of excretion of phosphates, introduces an important factor complicating the picture of phosphatemia in acidosis. See discussion in the section on nephritis.

later was found to be similarly reduced in the blood cells of patients with acute diabetic acidosis, and of infants suffering with severe acidosis due to gastroenteritis (51).

In acidotic conditions such as those just named, at least three closely related factors can be cited as probably influencing the decrease in concentration of diphosphoglycerate in the blood cells: namely, decreased pH of the blood, changes in concentration of chloride in the serum and cells, and a decreased concentration of total base in the cells. It has been pointed out (110) that if blood, incubated *in vitro*, be acidified to pH below 7.3 the diphosphoglycerate in the cells is rapidly decomposed and inorganic P is liberated—an effect on the glycolytic enzyme system which presumably is operative also *in vivo*. The exact relationship of the blood chlorides to changes in concentration of diphosphoglycerate in the cells is mainly a subject for conjecture. Since the relative distribution of Cl between serum and cells is closely correlated with the pH of the blood, an increased ratio $Cl_{cells} : Cl_{serum}$ can be considered merely an additional index of the acidotic condition (see 54). However, the striking reciprocal relationship that has been observed in a number of conditions between changes in concentration of Cl and diphosphoglycerate in the blood cells, apparently independent of the pH of the blood, suggests that the absolute concentration of Cl may itself influence the synthesis and decomposition of diphosphoglycerate. A decreased concentration of total base in the cells may be regarded as a factor influencing the concentration of diphosphoglycerate just as a decrease of total base in blood serum results perforce in a decrease of bicarbonate if the other anions are not reduced. This may be seen in diabetic acidosis, where much base is lost in the urine, and in the acidosis of gastroenteritis in infants where base is lost in diarrheal stools.

While the organic acid-soluble P compounds of the blood appear to be one source of the increased phosphaturia of acidosis, this source is relatively small compared with the total urinary phosphorus excretion. Acid-soluble P compounds of the soft tissues seem to be closely related to those of the blood and to be similarly affected by acidotic conditions. Data reported by Farquharson, Salter, Tibbets and Aub (37) on the proportions of nitrogen, calcium, and phosphorus in the urine of subjects during the rapid development of acidosis would indicate that the acid-soluble P compounds of the soft tissues were drawn upon before other stores for the mobilization of phosphorus. The decreases in concentration of the organic phosphates in the blood cells, constituting only a part of the general depletion of labile phosphorus reserves of the

body, thus appear to be an index of similar processes occurring in other tissues.

During recovery from acute severe diabetic acidosis, changes occurring in the concentration and distribution of acid-soluble P compounds in the blood appear to be closely correlated with the rate of restoration of the labile phosphorus reserves of the body to normal. This was discussed in connection with the case reported by Guest and Rapoport (51, chart 7). In the blood of that patient the restoration of the organic acid-soluble P to a normal concentration in the cells was slow compared with the restoration of the blood sugar, CO_2, and Cl, to normal. Insulin therapy was followed by a sharp fall in the concentration of inorganic P in both plasma and cells, and of adenosinetriphosphate in the cells, and the levels of both inorganic P and adenosinetriphosphate remained low until after the diphosphoglycerate reached a normal concentration in the blood cells (more than 4 days). After the first 24 hours practically no phosphorus was excreted in the urine during more than 7 days following symptomatic recovery of the patient. The reappearance of normal phosphaturia, after the distribution of phosphorus in the blood was restored to normal, was believed to indicate that a state of equilibrium had been reached between the demand for phosphorus in the tissues and the supply of phosphorus available.

The manifestations of acidosis, such as fatigue, dehydration, hyperpnea, etc., may be described as the result of concomitant pathologic processes in blood and tissues which mutually aggravate each other. Thus the rapid development of acidosis, due either to base deficit or to the accumulation of acids in the blood, is accompanied by losses of various electrolytes from the body, dehydration, changes in the blood such as decreased pH, shifts in Cl distribution, decomposition of diphosphoglycerate, etc. Excessive phosphaturia leading to depletion of the labile phosphorus reserves of the body may result in profound disturbances of tissue metabolism since the phosphoric esters in the tissues are concerned with many different cellular activities.

Haldane, Wigglesworth and Kay (56) stated that some of the ill effects of acidosis might be due to the depletion of the labile phosphorus reserves of the body, and suggested that in such conditions the administration of phosphate might be advantageous. We have studied the effects of the administration of phosphates in the treatment of three types of acidosis: namely, acidosis due to the ingestion of ammonium chloride (51, chart 5); diabetic acidosis (53); and acidosis due to gastroenteritis in infants (50; 51, chart 6). Although incomplete the results

suggest that in such conditions the administration of phosphates, by mouth and intravenously and in connection with other therapeutic procedures usually employed, may hasten symptomatic recovery and the restoration of a normal distribution of phosphorus in the blood.

Alkalosis. As pointed out in the discussion of glycolysis, reactions of the blood above pH 7.3 have been shown to favor esterification of inorganic P in the blood cells *in vitro.* Apparently no studies have been made of the influence of alkalosis alone on the distribution of organic phosphates in the blood *in vivo.* Haldane and co-workers (56) showed that alkalosis created by overbreathing resulted in a sharp fall in concentration of inorganic P in the blood (see also Holtz, 64) and diminished excretion of phosphates in the urine. It is probable, however, that increased alkalinity of the blood can produce only a small increase in the concentration of organic phosphates in the cells unless at the same time the concentration of chloride is decreased.

Hypochloremia. The concentration of organic acid-soluble P in the blood cells has been found to increase concomitantly with decreases in concentration of chloride in the blood cells of dogs following pyloric obstruction (4, 48), of dogs and rabbits following bilateral nephrectomy, and of dogs, rabbits and human beings in the terminal stages of nephritis (5, 48, 51, 54). In such conditions the increase of organic acid-soluble P may be practically all accounted for in the diphosphoglycerate fraction. With the hypochloremia in such conditions the blood may become more alkaline than normal (as with vomiting), or more acid (as in nephritis), or may maintain a normal pH. The importance of increases in the concentration of diphosphoglycerate in compensating for losses of Cl from the blood cells in such conditions has already been discussed.

Nephritis. Since much of the existing literature dealing with the phosphatemia of nephritis was reviewed by Peters and Van Slyke (105) in 1931, it seems unnecessary to repeat here their citations of this extensive literature. In their discussion of the significance of increased concentrations of inorganic P in the blood as a sign of failure of renal function, they pointed out that the inorganic P of the blood is abnormally high in cases of nephritis with gross retention of nitrogenous waste products, that the hyperphosphatemia parallels increases in blood creatinine rather closely, the blood urea less closely, and that considerable prognostic importance has been accorded to the concentration of phosphate in the blood serum in nephritis. They stated, however, that known facts did not justify an assumption that the hyperphos-

phatemia of nephritis could be due entirely to disability of the kidneys to excrete phosphates. With regard to hyperphosphatemia as a contributory cause of the acidosis of terminal nephritis, they cited evidence that while hyperphosphatemia in nephritis is usually accompanied by lowered plasma bicarbonate and acidosis, the increased phosphoric acid in the blood plays a less important part in producing acidosis than other combined acidogenic factors.

Present knowledge makes it difficult to estimate the relative importance of "retention" *per se* as a factor influencing the level of inorganic P in the blood in nephritis and other conditions attended with suppression of renal function. In such conditions, however, it appears that increased concentrations of both inorganic and organic phosphates in the blood are due to the combined effects, direct and indirect, of renal damage and of various extrarenal factors such as those mentioned in the preceding discussion of ionic equilibrium and glycolytic processes in the blood. The renal and extrarenal factors respectively may have more or less influence upon phosphatemia according to the type and severity and stage of the disease. In any case, the organic as well as the inorganic phosphates in the blood should be considered when evaluating the significance of hyperphosphatemia in nephritic acidosis.

Increased concentrations of organic acid-soluble P in the blood cells of patients suffering with nephritis and other renal disorders were reported by Byrom and Kay (20) in 1927. In such bloods they found the fraction of the ester P hydrolyzable by bone enzyme increased, and the non-hydrolyzable fraction decreased. Hoesch (63) reported somewhat similar data on the bloods of nephritic patients, showing the fractions of the organic acid-soluble P hydrolyzable and non-hydrolyzable by N HCl to be increased and decreased, respectively. Stearns and Warweg (129b) reported increases of organic acid-soluble P in the blood cells of children suffering with severe renal damage. In 1934 Ashley and Guest (5) found that the acute suppression of renal function that resulted from injections of diphtheria toxin and $HgCl_2$, and bilateral nephrectomy, led to increases of inorganic P in the blood plasma and cells and of organic acid-soluble P in the blood cells, accompanied by decreases of chloride. The increases in concentration of organic acid-soluble P in the cells occurring in such conditions later were accounted for by increases in the diphosphoglycerate fraction (51, 54).

Some of the effects of abrupt cessation of renal function are illustrated in chart 3 of Guest and Rapoport (51), representing changes found in the blood of a dog during 7 days following bilateral nephrectomy.

Those changes were: 1, progressive increase of non-protein nitrogen; 2, increase of inorganic P, somewhat slower at first than later; 3, increase of organic acid-soluble P in the cells, practically all accounted for by nearly equal increases in the diphosphoglycerate fraction; 4, decrease of HCO_3 and Cl in both serum and cells. The changes found in the blood samples drawn the last two days of this experiment correspond closely with those we have found repeatedly in blood samples drawn from patients in the terminal stages of chronic nephritis when renal function was reduced nearly to nil. A much more varied picture may be drawn, however, of changes which occur in the bloods of patients in different stages of acute and chronic nephritis. In table IV of a recent paper (54) are listed data on 4 blood samples from patients suffering with chronic nephritis with varying degrees of renal impairment and in different conditions of acidosis. In such patients normal, low, or high concentrations of organic acid-soluble P in the blood cells may accompany decreases of HCO_3 and pH of the serum. Such findings suggest that the phosphatemia of nephritis is subject to a balance between the effects of acidosis and phosphate retention, respectively favoring decreases and increases of organic acid-soluble P in the cells. Thus acidosis from any cause in a nephritic patient may result in a decreased concentration of phosphoric esters in the blood cells if the excretory function is adequate. If, however, phosphate excretion fails, the concentration of organic acid-soluble P in the cells may increase in spite of a lowered pH of the blood plasma. During periods of rapid change in the general condition of nephritic patients due to acute infections or other causes, we have occasionally observed large fluctuations in the relative proportions of adenosinetriphosphate and diphosphoglycerate in the blood, finding concentrations of adenosinetriphosphate as high as 30 mgm. per 100 cc. of cells. Such observations are in accord with the findings of Byrom and Kay (20), and of Hoesch (63), that the hydrolyzable fraction of the organic acid-soluble P in blood cells of nephritic patients was relatively increased, compared with the non-hydrolyzable fraction.

As has been described in other conditions, changes in concentration of various electrolytes in both plasma and cells occur concomitantly with changes in the distribution of phosphorus in nephritic bloods. Therefore the concentrations and distribution in serum and cells of the Cl, total base, etc., must be included in the consideration of factors which may influence the changes in distribution of phosphorus in the blood in any given case of nephritis.

Rickets. Although most studies of phosphatemia in rickets have been limited to the inorganic P of plasma or serum, there exists much evidence that changes in the concentration of organic acid-soluble P compounds of the blood cells are important in the chemical pathology of this condition. Iverson and Lenstrup in 1920 (65) and later Freudenberg (41) found an increase of total acid-soluble P in the whole blood of rachitic infants during the period of healing of rickets, and in 1925 Robison and Soames (116) found in the whole blood of rachitic rats a reduced concentration of phosphoric esters hydrolyzable by bone enzyme (see also Euler and Brandt, 35). Wilder (137) found increases of organic acid-soluble P in the blood cells of rachitic rats during the healing that occurred with fasting. A reduced concentration of readily hydrolyzable organic phosphate in the blood cells of rachitic infants was reported by Bakwin, Bodansky and Turner (7a) and similar findings on the blood of rachitic rats were reported by Jacobsen (66). Bakwin and co-workers later (7b) found the lowered concentration of organic acid-soluble P in the blood cells of rachitic infants to be partly accounted for by decreases in the readily hydrolyzable fraction, adenosinetriphosphate, and partly by decreases in the fraction not hydrolyzed by bone enzyme—a fraction they surmised would prove to be diphosphoglycerate. Stearns and Warweg (129a) found increases of ester P in the blood cells of three children during the healing stages of late rickets. Kay (72) and Skill and Kay (126) reported that various types of experimental rickets (including "Beryllium rickets") were associated with a great reduction in the concentration of phosphoric esters in the blood cells and in the liver as well as with decreased concentrations of inorganic P in the blood. Kay and Skill (76) reported also that recovery of rachitic rats was associated with a rapid increase in the phosphoric ester content of the blood cells. Kay's observations of parallel decreases of organic acid-soluble P in blood cells and in liver may be cited as important evidence that changes in the concentration of phosphoric esters in the blood cells constitute an index of the state of the labile phosphorus reserves of the whole body.

We have reported data (51, 109) showing that the development of rickets induced in rats by a high Ca low P diet was associated with decreases first of inorganic P and of adenosinetriphosphate and then of diphosphoglycerate in the blood cells. During the development of rickets the progressive decrease in concentration of diphosphoglycerate in the blood cells appeared to reflect an increasing deficiency of phosphorus in the rats. Healing of the rickets, induced by a minimum

healing dose of vitamin D, or by the addition of phosphate to the diet, or by fasting, was preceded by increases first of diphosphoglycerate and then of adenosinetriphosphate and inorganic P in the blood cells. A rapid increase in concentration of diphosphoglycerate in the blood cells following any of the procedures which induced healing appeared to be a sign of rapid mobilization of phosphorus in the body and of the availability of such phosphorus for transport to the calcifying bones.

Also of interest in this discussion of rickets are some unpublished observations we have made on the distribution of phosphorus in the bloods of monkeys in relation to intake of vitamin D and seasonal exposure to sunlight. A monkey that had been kept in the laboratory several years had developed great weakness—apparently the condition commonly designated "cage paralysis." The concentration of inorganic P in the blood was 1.1 mgm. per 100 cc., and that of organic acid-soluble P in the cells, 28 mgm. per 100 cc. Roentgenograms of the bones disclosed extreme osteoporosis. Vitamin D therapy without other change in the diet was followed by a rapid improvement in the general condition of the monkey and restoration of the blood phosphorus to normal levels. When we subsequently examined a number of apparently normal monkeys that had been kept in the laboratory one to two years, several were found to have low concentrations of both inorganic and organic phosphates in the blood and most of these had definite roentgenographic signs of rickets or osteoporosis. Low concentrations of inorganic and organic phosphates also were found in the bloods of supposedly normal young monkeys newly arrived in the laboratory during winter months, although roentgenographic studies of their bones not always revealed visible signs of rickets. The administration of vitamin D to these monkeys was followed by increases of the blood phosphorus to normal concentrations. Monkeys of the same laboratory kept out of doors during summer months were found to have normal concentrations of inorganic and organic phosphates in their bloods.

Overdosage effects of irradiated ergosterol. In 1933 Guest and Warkany (55) reported that the administration of large doses of irradiated ergosterol to normal rabbits was followed by large increases in the concentration of organic acid-soluble P in the blood cells. Later, we found these changes to be practically limited to the diphosphoglycerate fraction (49). Observations made in the course of the latter studies suggested that the changes in concentration of organic phosphates in the blood cells were secondary to effects of the irradiated ergosterol

on other body tissues. In exsanguination-replacement blood transfusion experiments, blood was exchanged between normal rabbits and rabbits intoxicated with high doses of irradiated ergosterol. In blood cells from normal rabbits, injected into intoxicated rabbits, the concentration of diphosphoglycerate quickly increased to a level previously determined in the intoxicated rabbit's own blood cells. In blood cells from intoxicated rabbits, injected into normal rabbits, the concentration of diphosphoglycerate fell quickly to the normal level previously determined in the recipient's own cells. That the diphosphoglycerate in the blood cells thus proved to be so labile *in vivo* under such conditions is another indication that its concentration is governed largely by the state of the labile phosphorus reserves of the body.

Because of these experimental observations, it was suggested (25) that changes in the distribution of phosphorus in the blood might afford a sign of approaching intoxication in patients treated with large doses of vitamin D for various reasons. Studies were subsequently made on the bloods of several adult arthritic patients who were given daily doses of 50,000 to 500,000 international units of vitamin D for varying periods of time. However, only slight changes were found in the distribution of acid-soluble P in the blood cells of these patients even when the titre of vitamin D in the blood serum rose to as high as 13,000 units per 100 cc. (132). No attempt was made to increase the daily doses of vitamin D given these patients to amounts causing more than mild symptoms of intoxication.

Parathyroid hormone. Various investigators studying relationships between the parathyroid glands and phosphorus metabolism have found that injections of parathyroid extract are followed within a few hours by a greatly increased excretion of phosphorus in the urine, with a concomitant decrease in the concentration of inorganic P in the blood (2, 42). These changes preceded the effects of this hormone on calcium metabolism—increased calcium excretion and increased concentration of calcium in the blood—effects which usually were not apparent earlier than 24 hours after the injection. Cantarow, Brundage and Housel (22) reported data on changes of organic acid-soluble P in the blood of dogs following injections of large doses of parathyroid extract showing that the organic acid-soluble P in the blood cells first decreased and later, with the development of dehydration and failure of renal function, increased. We have confirmed these observations and found that changes in the diphosphoglycerate accounted for the changes of organic acid-soluble P. Thus it appears that the first effect of large doses of

the parathyroid hormone is a mobilization of labile phosphorus reserves. With a decreased renal threshold for phosphate excretion (1, 28, 58), increased phosphaturia leads to a decrease in the concentration of organic acid-soluble P in the blood cells. Subsequently, the development of dehydration and failure of renal excretion (125) probably account for the increases of inorganic and organic acid-soluble phosphates in the blood.

Anemias. Byrom and Kay (21) found a high concentration of organic acid-soluble P in the blood cells of patients with pernicious anemia and leukemia. Warweg and Stearns (133) studied the distribution of phosphorus in the blood of a patient during recovery from a severe anemia caused by prolonged gastro-intestinal hemorrhages. During the initial period of recovery the organic acid-soluble P in the blood cells increased to a high concentration and then decreased gradually as the cell count and hemoglobin content of the blood approached normal. Kinzel (82) reported a decrease of organic acid-soluble P in the blood cells of rabbits subjected to bleeding and treatment with phenylhydrazine—results which are not in accord with the findings of other investigators who made similar experiments. Malan (92) reported increased concentrations of organic acid-soluble P, with the appearance of considerable amounts of nucleoprotein, in the blood cells of anemic sheep and cattle. In newborn infants with severe jaundice, anemia, and erythroblastosis neonatorum, we have observed rapid increases in the concentration of organic acid-soluble P in the blood cells occurring during the first few days of life. Following recovery from these conditions, the organic acid-soluble P decreased to a normal concentration in the cells within a few days.

SUMMARY

In this review attention is directed mainly to available information concerning the functions of the organic acid-soluble phosphorus compounds in mammalian bloods, and to changes in the concentration of these compounds in the blood observed in different conditions.

The distribution of the phosphoric esters in normal blood varies greatly in different species, and among normal individuals of a species the distribution may vary according to age, type of diet, and other factors.

As non-diffusible anions in the cells, these compounds are important factors in the electrolyte equilibrium of the blood. Inasmuch as the phosphoric esters are synthesized and decomposed through reactions

of the glycolytic cycle, the enzymatic processes of glycolysis have an important dynamic rôle in the maintenance of the electrolyte equilibrium of the blood.

The greatest changes in concentration of organic acid-soluble phosphorus in the blood cells in pathologic conditions are usually in the diphosphoglycerate fraction, at least in human, dog, rabbit, and rat bloods, the species most studied. This fraction is decreased with phosphorus deficiency in rickets and in various types of acidosis in subjects with adequate renal function, and increased after pyloric obstruction, after suppression of renal function, and in some other conditions. The significance of the changes in distribution of phosphorus in the blood in such conditions is discussed with regard to various metabolic disturbances, and to the state of the labile phosphorus reserves in the body.

REFERENCES

(1) Albright, F., W. Bauer, D. Claflin and J. R. Cockrill. J. Clin. Investigation **11:** 411, 1932.

(2) Albright, F., W. Bauer, M. Ropes and J. C. Aub. J. Clin. Investigation **7:** 139, 1929.

(3) Anderson, H. D. and C. A. Elvehjem. J. Biol. Chem. **134:** 217, 1940.

(4) Andrus, W. deW., G. M. Guest, R. F. Gates and A. Ashley. J. Clin. Investigation **11:** 475, 1932.

(5) Ashley, A. and G. M. Guest. J. Clin. Investigation **13:** 219, 1934.

(6) Bajev, A. A. Compt. rend. acad. sci. U.R.S.S. **4:** 69, 1934; (Chem. Abst. **29:** 2221); Biokhimiya **2:** 454, 1937.

(7) Bakwin, H., O. Bodansky and R. Turner. Proc. Soc. Exper. Biol. and Med. **29:** 1238, 1931a.
Proc. Soc. Exper. Biol. and Med. **36:** 365, 1937b.

(8) Barer, A. P. J. Clin. Investigation **10:** 507, 1931.

(9) Barrenscheen, H. K. and H. Beneschovsky. Biochem. Ztschr. **265:** 159, 1933; Biochem. Ztschr. **276:** 147, 1935.

(10) Barrenscheen, H. K. and K. Braun. Biochem. Ztschr. **231:** 144, 1931.

(11) Barrenscheen, H. K. and W. Filz. Biochem. Ztschr. **240:** 409, 1931.

(12) Barrenscheen, H. K. and K. Hübner. Biochem. Ztschr. **229:** 329, 1930.

(13) Barrenscheen, H. K. and B. Vasarhelyi. Biochem. Ztschr. **230:** 330, 1931.

(14) Baumann, C. A. and F. J. Stare. Physiol. Rev. **19:** 353, 1939.

(15) Bomskov, C. Ztschr. f. physiol. Chem. **210:** 67, 1932.

(16) Braunstein, A. E. Biochem. Ztschr. **272:** 21, 1934.

(17) Braunstein, A. E. and B. A. Severin. Biochem. Ztschr. **276:** 359, 1935.

(18) Buell, M. V. J. Biol. Chem. **108:** 273, 1935.

(19) Byrom, F. B. Brit. J. Exper. Path. **10:** 10, 1929.

(20) Byrom, F. B. and H. D. Kay. Brit. J. Exper. Path. **8:** 429, 1927.

(21) Byrom, F. B. and H. D. Kay. Brit. J. Exper. Path. **9:** 72, 1928.

(22) CANTAROW, A., J. T. BRUNDAGE AND E. L. HOUSEL. Endocrinology **21:** 368, 1937.
(23) CORI, G. T. AND C. F. CORI. J. Biol. Chem. **94:** 561, 1931.
(24) DISCHE, Z. Biochem. Ztschr. **274:** 51, 1934; **280:** 248, 1935; Naturwissenschaften **22:** 417, 776, 855, 1934; **23:** 311, 1935; **24:** 462, 1936; **25:** 650, 1937; Enzymologia **1:** 288, 1936.
(25) DORST, S. E., G. M. GUEST, S. RAPOPORT AND J. WARKANY. J. Clin. Investigation **18:** 478, 1939.
(26) EGGLETON, G. P. AND P. EGGLETON. J. Physiol. **68:** 193, 1929.
(27) EISENMAN, A. J., L. OTT, P. K. SMITH AND A. W. WINKLER. J. Biol. Chem. **135:** 165, 1940.
(28) ELLSWORTH, R. J. Clin. Investigation **11:** 1011, 1932.
(29) ENGELHARDT, W. A. Biochem. Ztschr. **227:** 16, 1930; **251:** 343, 1932.
(30) ENGELHARDT, V. A. AND A. A. BAEV. Biokhimiya **1:** 113, 1936; (Chem. Abst. **30:** 6051); Compt. rend. acad. sci. U.R.S.S. **2:** 331, 1936 (Chem. Abst. **30:** 7661).
(31) ENGELHARDT, W. A. AND A. E. BRAUNSTEIN. Biochem. Ztschr. **201:** 48, 1928.
(32) ENGELHARDT, V. A. AND A. I. KOLOTILOVA. Trans. Physiol. Inst. Leningrad **16:** 13, 1936 (Chem. Abst. **32:** 5885).
(33) ENGELHARDT, W. A. AND M. LJUBIMOWA. Biochem. Ztschr. **227:** 6, 1930.
(34) ENGELHARDT, V. A. AND M. N. LJUBIMOVA. Compt. rend. acad. sci., U.R.S.S. **2:** 329, 1936 (Chem. Abst. **30:** 7661).
(35) VON EULER, H. AND K. M. BRANDT. Ztschr. physiol. Chem. **240:** 215, 1936.
(36) FALCON-LESSES, M. Arch. Int. Med. **39:** 412, 1927.
(37) FARQUHARSON, R. F., W. T. SALTER, D. M. TIBBETS AND J. C. AUB. J. Clin. Investigation **10:** 221, 1931.
(38) FISKE, C. H. Proc. Natl. Acad. Sci. **20:** 25, 1934.
(39) FISKE, C. H. AND Y. SUBBAROW. J. Biol. Chem. **66:** 375, 1925.
(40) FORBES, E. B. AND M. H. KEITH. A review of the literature of phosphorus compounds in animal metabolism. Wooster, Ohio Agricultural Experiment Station, Technical Series Bull. **5:** p. 510, 1914.
(41) FREUDENBERG, E. Ztschr. f. Kinderheilk. **57:** 427, 1936.
(42) GOADBY, H. K. AND R. S. STACEY. Biochem. J. **28:** 2092, 1934.
(43) GREEN, H. H. AND E. H. MACASKILL. J. Agric. Sci. **18:** 384, 1928.
(44) GREENBERG, D. M. Ann. Rev. Biochem. **8:** 269, 1939.
(45) GREENWALD, I. J. Biol. Chem. **14:** 269, 1913.
(46) GUEST, G. M. J. Clin. Investigation **11:** 555, 1932.
(47) GUEST, G. M. J. Clin. Investigation **11:** 571, 1932.
(48) GUEST, G. M. AND W. DEW. ANDRUS. J. Clin. Investigation **11:** 455, 1932.
(49) GUEST, G. M. AND S. RAPOPORT. J. Biol. Chem. **124:** 599, 1938.
(50) GUEST, G. M. AND S. RAPOPORT. Trans. Soc. for Pediat. Res., Am. J. Dis. Child. **56:** 942, 1938.
(51) GUEST, G. M. AND S. RAPOPORT. Am. J. Dis. Child. **58:** 1072, 1939.
(52) GUEST, G. M. AND S. RAPOPORT. Publication no. 13 of Am. Assn. for Advancement of Science; Symposium on Blood, Heart and Circulation, Washington, D. C., Science Press, 1940, p. 55.
(53) GUEST, G. M. AND S. RAPOPORT. Proc. Am. Pediat. Soc., Am. J. Dis. Child. **60:** 450, 1940.

(54) GUEST, G. M. AND S. RAPOPORT. J. Lab. and Clin. Med. **26:** 190, 1940.
(55) GUEST, G. M. AND J. WARKANY. J. Biol. Chem. **100:** 445, 1933.
(56) HALDANE, J. B. S., V. B. WIGGLESWORTH AND C. E. WOODROW. Proc. Roy. Soc. London, S. B. **96:** 1, 1924–25.
(57) HALPERN, L. J. Biol. Chem. **114:** 747, 1936.
(58) HARRISON, H. E. AND H. C. HARRISON. Trans. Soc. Pediat. Res., Am. J. Dis. Child. **60:** 994, 1940. J. Clin. Investigation **20:** 47, 1941.
(59) HENRIQUES, V. AND S. L. Ørskov. Skandinav. Arch. f. Physiol. **82:** 86, 1939.
(60) HEVESY, G. Enzymologia **5:** 138, 1938.
J. Chem. Soc., p. 1213, July, 1939.
Ann. Rev. Biochem. **9:** 641, 1940.
(61) HEVESY, G. AND A. H. W. ATEN. Kgl. Danske Videnskab. Selskab. Biol. Medd. **14:** no. 5, 1939.
(62) HEVESY, G. AND L. HAHN. Ibid., **15:** no. 7, 1940.
(63) HOESCH, K. Ztschr. f. klin. Med. **121:** 305, 1932.
(64) HOLTZ, F. Ztschr. f. physiol. Chem. **194:** 76, 1931.
(65) IVERSON, P. AND E. LENSTRUP. Forhandl. Første Nord. Kong. Paediat. **89**, 1920.
(66) JACOBSEN, E. Biochem. Ztschr. **263:** 313, 1933.
(67) JOHN, H. J. Ann. Clin. Med. **3:** 667, 1925.
(68) JOST, H. Ztschr. f. physiol. Chem. **165:** 171, 1927.
(69) KAY, H. D. Biochem. J. **18:** 1133, 1924.
(70) KAY, H. D. Biochem. J. **19:** 447, 1925.
J. Physiol. **65:** 374, 1928.
(71) KAY, H. D. Brit. J. Exper. Path. **11:** 148, 1930.
(72) KAY, H. D. J. Biol. Chem. **99:** 85, 1932.
(73) KAY, H. D. Ann. Rev. Biochem. **1:** 187, 1932a.
Ann. Rev. Biochem. **3:** 133, 1934b.
(74) KAY, H. D. AND F. B. BYROM. Brit. J. Exper. Path. **8:** 240, 1927.
(75) KAY, H. D. AND R. ROBISON. Biochem. J. **18:** 1139, 1924.
(76) KAY, H. D. AND D. I. SKILL. Biochem. J. **28:** 1222, 1934.
(77) KERR, S. E. J. Biol. Chem. **117:** 227, 1937.
(78) KERR, S. E. AND A. ANTAKI. J. Biol. Chem. **121:** 531, 1937.
(79) KERR, S. E. AND M. E. BLISH. J. Biol. Chem. **98:** 193, 1932.
(80) KERR, S. E. AND L. DAOUD. J. Biol. Chem. **109:** 301, 1935.
(81) KIESSLING, W. Biochem. Ztschr. **273:** 103, 1934.
(82) KINZEL, B. Ztschr. f. Kinderheilk. **59:** 160, 1938.
(83) KOLOTILOVA, A. I. AND W. A. ENGELHARDT. Biokhimiya **2:** 387, 1937.
(84) LAWACZECK, H. Biochem. Ztschr. **145:** 351. 1924.
(85) LYUBIMOVA, M. N. Biokhimiya **2:** 367, 1937.
(86) LOHMANN, K. Biochem. Ztschr. **202:** 466, 1928.
(87) LOHMANN, K. Biochem. Ztschr. **254:** 381, 1932.
(88) LOHMANN, K. Ann. Rev. Biochem. **7:** 125, 1938.
(89) LUNDSGAARD, E. Ergebn. d. Enzymforsch. **2:** 179, 1933.
(90) MAIZELS, M. AND J. L. H. PATERSON. Biochem. J. **31:** 1642, 1937.
(91) MALAN, A. I. J. Agric. Sci. **18:** 397, 1928.
(92) MALAN, A. I. J. Agric. Sci. **18:** 401, 1928.
(93) MARTLAND, M. Biochem. J. **19:** 117, 1925.
(94) MARTLAND, M. AND R. ROBISON. Biochem. J. **20:** 847, 1926.

(95) McCay, C. M. J. Biol. Chem. **90:** 497, 1931.
(96) Meier, R. and E. Thoenes. Arch. f. exper. Path. u. Pharmakol. **161:** 119, 1931.
Deutsch. Arch. f. klin. Med. **172:** 160, 1931.
(97) Meyerhof, O. Biochem. Ztschr. **246:** 249, 1932.
(98) Meyerhof, O. Ergebn. d. Physiol. **39:** 10, 1937.
Helv. Chim. Acta **18:** 1030, 1936.
New England J. Med. **220:** 49, 1939.
(99) Michel-Durand, E. Bull Soc. Chim. Biol. **20:** 413, 1938.
(100) Morgulis, S. and J. D. Munsell. Biochem. Ztschr. **278:** 89, 1935.
(101) Needham, D. M. Enzymologia **5:** 158, 1938.
(102) Negelein, E. and H. Broemel. Biochem. Ztschr. **301:** 135, 1939.
(103) Nissen, H. Ztschr. f. Kinderheilk. **57:** 289, 1936.
(104) Parnas, J. K. Ergebn. d. Enzymforsch. **6:** 57, 1937.
(105) Peters, J. P. and D. D. Van Slyke. Quantitative clinical chemistry. Baltimore, Williams & Wilkins, 1931. Vol. I, Interpretations, p. 1090.
(106) Rapoport, S. Biochem. Ztschr. **289:** 406, 1937.
(107) Rapoport, S. Biochem. Ztschr. **289:** 411, 416, 1937.
(108) Rapoport, S. J. Biol. Chem. **135:** 403, 1940.
(109) Rapoport, S. and G. M. Guest. J. Biol. Chem. **126:** 749, 1938.
(110) Rapoport, S. and G. M. Guest. J. Biol. Chem. **129:** 781, 1939.
(111) Rapoport, S. and G. M. Guest. J. Biol. Chem. **131:** 675, 1939.
(112) Rapoport, S. and G. M. Guest. J. Biol. Chem. **138:** 269, 1941.
(113) Robison, R. Biochem. J. **17:** 286, 1923.
(114) Robison, R. Significance of phosphoric esters in metabolism. New York University Press, 1932.
(115) Robison, R. Ann. Rev. Biochem. **5:** 181, 1936.
(116) Robison, R. and K. M. Soames. Biochem. J. **19:** 153, 1925.
(117) Roche, A. and J. Roche. Compt. rend. Soc. de Biol. **96:** 361, 1927.
(118) Roche, A. and J. Roche. Bull. Soc. chim. biol. **11:** 549, 1929.
(119) Roche, A. and J. Roche. Bull. Soc. chim. biol. **15:** 520, 1933.
(120) Rona, P. and K. Iwasaki. Biochem. Ztschr. **184:** 318, 1927.
(121) Rubin, S. H. J. Biol. Chem. **126:** 679, 1938.
(122) Schmidt, C. L. A. and D. M. Greenberg. Physiol. Rev. **15:** 297, 1935.
(123) Schuchardt, W. and A. Vercellone. Biochem. Ztschr. **272:** 437, 1934; **275:** 261, 1935; **276:** 280, 1935.
(124) Severin, V. A. Biokhimiya **2:** 60, 1937.
(125) Shelling, D. H., L. Kajdi and L. Guth. Endocrinology **22:** 225, 1938.
(126) Skill, D. I. and H. D. Kay. Biochem. J. **28:** 1228, 1934.
(127) Solomon, R. Z., P. M. Hald and J. P. Peters. J. Biol. Chem. **132:** 723, 1940.
(128) Stearns, G. and E. Warweg. J. Biol. Chem. **102:** 749, 1932.
(129) Stearns, G. and E. Warweg. Am. J. Dis. Child. **49:** 79, 1935a; **50:** 1164, 1935b.
(130) Van Slyke, D. D., H. Wu and F. C. McLean. J. Biol. Chem. **56:** 765, 1923.
(131) Warburg, O., F. Kubowitz and W. Christian. Biochem. Ztschr. **242:** 170, 1931.

(132) WARKANY, J., G. M. GUEST AND F. J. GRABILL. J. Lab. and Clin. Med. (in press).
(133) WARWEG, E. AND G. STEARNS. J. Clin. Investigation **13**: 411, 1933.
(134) WARWEG, E. AND G. STEARNS. J. Biol. Chem. **115**: 567, 1936.
(135) WATSON, C. J. The porphyrins and diseases of the blood, in Symposium on the blood and blood-forming organs. University of Wisconsin Press, 1939, p. 14.
(136) WATSON, C. J. AND W. O. CLARK. Proc. Soc. Exper. Biol. and Med. **36**: 65, 1937.
(137) WILDER, T. S. J. Biol. Chem. **81**: 65, 1929.

THE NUTRITION OF THE FETUS

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Numerous factors influence the healthy development of the embryo. They include the maternal supply of foodstuffs adequate to the fetal requirements both in regard to quantity and quality, the sources from which they are derived and their utilisation in the peculiar metabolism of growth and finally their modifications by species, heredity and disease.

The assessment of the state of fetal nutrition is difficult; pre-natal estimations of nutritional condition are difficult to make so long as the fetus is in the uterus. Obstetrical and radiological examinations and the assessment of maternal health are the only methods available, but their results are limited and relatively crude.

The post-natal indices include birth weight, vigour and post-natal growth, and of these the birth weight is probably the best at our disposal in the absence of obvious disease. In animals the greater the size at birth the better the nutrition but while this may be so in the human the complication of the relatively greater size of the human fetal cranium presents obstetric difficulties not encountered in animals. Strength and development of muscle is impossible to assess quantitatively in the newborn as are differences of weight due to bone, muscle and fat. Since, however, muscle and bone are the heaviest tissues, these if well developed conduce to a higher weight at birth. Although the birth weight is only a rough measure of nutritional state it does appear to give an indication of the future post-natal development and there is ample evidence to show that the rate of growth after birth is a measure of the condition before or at birth.

Before considering the metabolic development in the uterus it is necessary to outline briefly the comparative physiology of the fetus since most of the work has been performed in animals and it is not always possible to argue from one species to another, still less to man.

Comparative physiology. The Mammalia are divided into three main nutritional groups, the Monotremata (Echidna and Ornithorhynchus),

the Marsupialia and all other members of the Eutheria. In the first group an egg is laid, the yolk of which supplies the earliest nourishment (telolecithal). After laying it is carried on the maternal abdomen and the young, when hatched, feeds at its mother's nipples. In the Marsupialia, the young are born immature and are carried at the maternal hypogastric nipples. The ovum before birth is alecithal and the foodstuffs are derived by a yolk sac placenta. In the remaining Eutheria the foodstuffs are derived first from the residues of the discus proligerus and zona radiata (prochorion), later from the yolk sac placenta and finally by the allantoic vessels from either an allantoic placenta or a chorionic placenta.

The yolk sac varies with the species. It is large in the Rodentia, smaller in the Ungulata especially in comparison with the allantois. In the Insectivora its vessels penetrate the trophoblast but in the anthropoid apes and Man it is small and never leaving the body it never reaches the trophoblast (extra-embryonic ectoderm).

The allantois is the main source of nourishment in the Eutheria. Its mesodermal sac grows out from the entodermal hind gut in all mammals except Tarsius and the Primata where it merely forms a small outgrowth into the body stalk. The Primata have a chorionic placenta, that is, there is no allantoic sac entering it, but the allantoic (later called the umbilical) vessels grow into the mesodermal body stalk to the chorion composed of mesoderm and ectoderm (trophoblast) and thrown up into chorionic villi with a core of vessels within the villus.

The fetal portion of the placenta is derived from the trophoblast and the maternal part from the decidua. In the fully formed placenta there are six main tissues intervening between the fetal blood and the maternal blood, namely, the endothelium of the maternal blood vessels, the connective tissue or stroma of the decidua containing glands, the epithelium covering this and apposed to the fetal chorion, the chorion itself derived from the trophoblast, the connective tissue of the fetal villi and lastly the endothelium of the fetal vessels. The most characteristic fact about the placenta in all species is that it always undergoes degenerative changes toward the end of uterine life. In consequence there are hemorrhages of the maternal blood, which may stagnate and autolyse; the fetal chorionic villi appear to absorb as foodstuff the glandular decidual secretion, the autolysed uterine tissue and hemorrhages. This necrosis progresses to different stages in different species. Grosser (1925) classifies the placenta according to the degree of degen-

eration and his classification is of most use in considering the layers across which foodstuffs have to transfuse. They are named after the maternal tissue apposed to the fetal tissue. There are four main types:

1. Epithelio-chorial. No necrosis. Decidual epithelium apposed to chorion: in the pig and horse.

2. Syndesmo-chorial. Decidual epithelium disappeared; seen in ruminants.

3. Endothelio-chorial. Gross degeneration and hemorrhages of the maternal connective tissue; in carnivora.

4. Hemo-chorial. Chorionic villi immersed in maternal blood; in rodents, apes and the human.

Mossman (1926) states the fact that in the rat and the rabbit the chorion may degenerate yielding only the fetal endothelium between the two bloods (hemo-endothelial). In the hemo-chorial types the blood does not stagnate and autolyse but circulates as in the splenic sinuses.

The embryological foodstuffs. This term is used to cover those materials in the ovum or uterus which do not come directly from the maternal diet. It includes:

1. The yolk of the egg in the Monotremata. Histologically it resembles that of the Sauropsida but there do not appear to be any recent analyses available.

2. The milk of the Marsupialia and the Monotremata. Wood Jones (1939) provides an analysis of the milk of the monotreme echidna. The outstanding fact is the very high percentage of solids and of the fat content.

Specific gravity	1.023
Total solids	36.75 per cent
Casein (?) (pH 4.7)	8.4 per cent
Albumen and globulin	2.9 per cent
Undetermined nitrogen	0.34 per cent
Lactose	2.81 per cent
Fat (Sapon. value 0.192)	19.62 per cent
Pigment	Little or none
Ash	0.78 per cent

3. The prochorion.

4. The embryotrophe. This includes the secretion of the uterine glands, and also the necrotic residues derived from the decidua and maternal hemorrhages. It is of greatest importance in the Ungulata. Analysis was made by Gamgee (1864).

The principal components are:

1. Cellular elements—blood cells and desquamate decidua.
2. Fluid elements—blood, lymph and glandular secretion.

The chief chemical compounds are proteins (10 per cent); glycogen and traces of fat (mainly intracellular); pigments which include hemoglobin and uteroverdin, a green derivative of hemoglobin peculiar to the carnivorous placenta; salts and crystals especially calcium oxalate. The phagocytic trophoblast cells ingest these compounds so that they are visible in the chorionic villi. The evidence for the chemistry of the embryotrophe is almost entirely histological.

The increase in fetal weight. The rates of deposition differ for different substances and at different ages. More than half the weight is deposited in the last six to eight weeks when there is accelerated deposition of all materials. For this reason a premature infant will put on weight more rapidly than a full term child. Water deposition is pronounced but that of solids is still more rapid, so that the water content of the body drops from 96 per cent at the end of the 2nd month to 74.5 per cent at term. Considering the dry weight, Needham has shown analogies with the bird in that the solid materials are neither deposited regularly during fetal life nor do they increase in quantity with a crescendo effect in the last two months but rather does one find variations in the percentage deposition signifying different rates of anabolism at different ages. The ash as a percentage of the dry weight rises till the 4th month (human fetus) and then remains constant, the fat shows a steady rise in the last quarter, whereas the protein has a peak at the 7th month and actually decreases in the subsequent period (as a percentage of the dry weight).

The daily demands of the fetus are shown in table 1.

The source of these materials may be the maternal tissues or the maternal diet. The relative utilisation of these two sources is not known nor is it possible to state in what proportion the mother's food goes to her own basal endogenous metabolism, to new tissue formation in her body or to fetal tissue formation.

Analyses have been made of the relation between fetal age and fetal growth by a number of workers using mathematical methods (Minot, 1891, 1908; Robertson, 1923; McDowall, Gates and McDowall, 1930; Brody, 1928; Schmalhausen, 1928). Their varying methods (logarithmic plotting, differentiated analysis, etc.) have yielded interesting results, the most important being some success in demonstrating that rate of growth can depend on the velocity of the catalysing reactions

concerned and that at different ages the changes in growth rate are controlled by new reactions with different velocities. The data available do not however permit any sweeping conclusion; it is not possible to consider the problem in the limits of this review but full discussions can be found in Needham (p. 368) and in Brody (1928).

The birth weight. Rubner (1908) first showed that in all species except man, the birth weight is proportional to the gestation time. In man it is below the expected weight. The reason for this is unknown, though it is as well to point out that the human has a disproportionately big head, especially in relation to the pelvic outlet.

Apart from this the most important factor is the food supply of the mother. Maternal overfeeding appears to have no effect on the birth weight but to increase the maternal weight in the sheep (Hammond, 1933). Moderate underfeeding has no effect in gilts (Hogan, 1928) nor in the sheep where however it depletes the maternal reserves and results in loss of weight (Hammond, 1933); and in the cow Eckles (1919) has found the same effect and regards breed as of greater importance. The same may be the explanation of the observations on birth weights in Central Europe in the 1914–19 War. On the other hand both Prochovnik, (1899 and 1901), and Jonen (1932) claim that restricted diet lowers the human birth weight, as also do Acosta-Sison and Calang (1931) in the Philippine Islands. In rats (Zuntz, 1924), guinea pigs (Paton, 1903) and rabbits (Reeb, 1905) underfeeding in pregnancy gives low birth weights, and Hammond (1933) has shown in sheep that the birth weight is markedly lowered if the underfeeding is so pronounced as to markedly depress the maternal plane of nutrition. Hammond's results as to moderate feeding in sheep depleting the maternal

TABLE 1

Composition of the human fetus in grams

	BODY	WATER	PROTEIN	FAT	ASH
End of 5th lunar month	300	260	22	3.5	1.5
End of 7th lunar month	1,000	800	100	25.0	30
End of 10th lunar month (birth)	3,200	2,420	400	350.0	90
Average of daily deposition:					
1. Throughout fetal life	11.4	8.6	1.4	1.25	0.32
2. In last 3 lunar months	26.2	19.3	3.57	3.87	0.71
3. In last month	35.7	23.6	6.4	5.0	2.0

reserves are confirmed in the human where it is known both loss of weight and loss of specific materials such as calcium, phosphorus, iron and vitamin D can occur with shortage of these or of calories or protein in the diet (see the later sections of this review and also Garry and Stiven, 1936).

The influence of the maternal food supply on the fetal development and birth weight is modified by competing metabolisms, notably the mother's own metabolism and that of twins, triplets or a litter. Hammond (1933) showed that the birth weight of single lambs was 29 per cent heavier, and that of individual triplets 9 per cent lighter than that of members of the twin pairs. The size of young at birth is inversely proportional to the number in litters of pigs, guinea pigs and rabbits. In addition if the ewe before lambing be ill-nourished then the birth weight of individual twins and triplets are less and the difference between their weights and that of single lambs at birth is proportional to the degree of malnutrition of the mother. Put differently the ratio

$$\frac{\text{Weight of single lambs}}{\text{Weight of individual twin lambs}}$$

was proportional to the nutritive impairment. This ratio is normally greater for female lambs than for male lambs from which he infers rams have a better chance of survival than ewe lambs and that in those species where the ratio is small there is a better chance of survival than in other species. There is no direct evidence of how this comes about or of the effects of male or female sex hormones on the growth of lambs in the uterus.

Hammond (1933) has also shown in the sheep that the birth weight is dependent on the ewe weight at birth. This in turn depends on 1, her nutritive condition: if deficient she is competing with her fetus; 2, her age: if still growing she is competing again with her young; 3, the species. He showed that the diet in the last weeks of pregnancy influenced the birth weight, summer lambs being heavier than early spring lambs. Toverud (1933) has shown in humans that the infant birth weight is highest in August and lowest in January. Vitamin A concentrates administered to pregnant cattle have been shown to increase the birth weight of the calves (Massachusetts 1938). The age of the ewe influences her birth weights in that her lambs are heavier the later the pregnancy. The same fact is well known in connection with child marriages where the birth weights (e.g., in India) are less than with adult marriages.

The genetic factor plays an important part. Eckles (1919) showed in experiments with Jersey and Holstein cows that breed was an important factor in influencing birth weight. Hammond (1933) notes the same thing in sheep. Walton and Hammond (1938) have also crossed a Shire mare with a Shetland horse, and a Shetland mare with a Shire horse. The birth weight of the foals was determined solely by the mare. The paternal influence only evinced itself in growth after birth, but even then the influence of the maternal breed was always predominant. They discuss three possible modes of maternal influence on fetal growth. They are first, cytoplasmic inheritance, in which the ovum receives a quota of growth regulating substance from the mother proportional to her size. There was some evidence of this in the mouse (Marshak, 1936). It does not however explain the results after birth nor those of Greene (1931) in which mouse cross-breed curves do meet in time. Hammond's second possibility is the bigger blood supply in the uterus of the bigger breed. This he excludes because in the rabbit a certain number of young in one horn are not smaller than the same number distributed in both horns. A further possibility is that there is maternal control of the fetal nutrition. This he excludes as inadequate to explain the results since all the evidence suggests that the fetus has priority over the mother and only extremes of maternal malnutrition affect fetal birth weights. By a process of exclusion he infers that a maternal growth-promoting substance (hormone) correlates the fetal and maternal development.

The birth weight influences the after growth for long periods after birth. It is considered in regard to animals by Marshak and Greene in the papers referred to above. Illingworth (1939) has drawn attention to the effect of low birth weight in humans and shown that it may under modern conditions of civilisation result in failure to catch up to normal weights. Hess (1927) has, however, shown that these underweight infants may catch up in four years with special care in feeding.

The rôle of the placenta. The placental activities fall under five heads: 1, its secretory capacity; 2, its storage capacity; 3, its endocrine activity; 4, its transfusion capacity; 5, its chemical composition. All these aspects are adequately covered by Needham's review except the endocrine activity which was, however, very fully reviewed in 1938 by Newton and will be referred to again later.

It is important to point out however that it is physiological for the placenta to degenerate in late uterine life (Marshall, 1922). This is exemplified in the placenta weight of the goat (Elliott, Hall and Hug-

gett, 1934). At the 10th week it weighed 354 grams, at the 16th 870 grams, at the 18th it decreased to 658 grams and at the 20th week was 520 grams. Harris (1933) reviews the human placental changes. At the 22nd week it is at its peak of fruition after which the fetal vascular endothelium begins to proliferate but the placental volume, the fetal blood flow through it and the bulk of the amniotic fluids are all still increasing till the 32nd week: meanwhile, however, obliterative endarteritis, placental infarction and calcification are progressing and after the 32nd week the circulating blood volume in the placenta decreases. At term therefore the amount of functional placenta is diminished, the placenta membrane is reduced to that of the hemo-chorial type. This results in increased permeability and decreased functional activity. The S. pallidum can be recovered from the human embryo after the 6th month but has never been recovered before the senescent changes are present (Harris, 1929). Needham in a more general way concludes bacteria only pass if pathological changes have occurred (p. 1503). Mason, Dalling and Gordon (1930) showed antitoxins cannot traverse the epithelio-chorial placenta of the Ungulata. Ratner, Jackson and Gruehl (1927) have shown that antitoxins can, however, transfuse the hemochorial placenta of the guinea pig. Corey (1932) has shown that insulin passes from the fetus to the mother in the last third of pregnancy.

The gross weight of the healthy placenta varies between one-fourth and one-twelfth of the fetal weight at term. The chances of survival of an unhealthy or under-nourished fetus are greater with a large ratio placenta. Excessive placental destruction by infarcts is associated with conditions of high fetal mortality.

It is perhaps best to point out here that when one speaks of a substance diffusing across the placental membrane or of a difference in concentration on two sides of the membrane readily allowing diffusion one is really avoiding a correct explanation of the action by the use of a phrase. Transfusion as we know it and even simple diffusion may involve chemical combinations (Verzar's phosphorylation theories—(1936) complex physico-chemical changes, e.g., the action of the bile salts in the intestine—(Mellanby, 1927) or the effect of the red cell membrane (Danielli, 1940), to mention a few instances). We know little or nothing about the chemical or physical forces involved at the placental membrane. Cunningham (1920) has shown that its properties differ at the epithelial and endothelial layers but very little beyond that work has been performed.

Carbohydrate metabolism in the placenta and fetus. Claude Bernard (1859) showed that rabbit's blood sugar was fructose and that it was also present in the amniotic and allantoic fluids. Since then it has been shown that some species have glucose and not fructose: for example, the goat amniotic sugar is glucose (Huggett, 1929). Dickens and Greville (1932) (Warburg technique) showed that all embryonic tissues anaerobically converted glucose to lactic acid but only some formed the acid from fructose. Of these only the outer membranes of the rat and guinea pig and allantois of the rabbit attacked fructose and glucose equally. The placentae of the rabbit, guinea pig, rat and mouse had hardly any effect on fructose: the same applied to the tissues of the embryo proper: human full-time placenta was less active against fructose than against glucose and embryo tissues (rat and rabbit) had no action on fructose even though it was present in the fetal blood. The whole problem of fructose in the fetus is curious and unsolved. This work was the logical extension of the work of Warburg and his school on glycolysis under aerobic and anaerobic conditions. They showed that embryonic rat tissues only caused glycolysis under anaerobic conditions, practically not at all aerobically and never in vivo, indicating that its respiration is adequate to prevent glycolysis (distinction from the rapid growing tumour) (Warburg, 1930; Negelein, 1925).

The fetal blood sugar is very constant at any one age but rises towards 100 mgm. at full term (Snyder and Hoskins, 1928). In twins it is in each less than the maternal blood sugar (Slemons, 1919; Lawrence, 1929). Lawrence (1940) also notes a case where the maternal B.S. was 180 mgm., the umbilical vein sugar 125 mgm. and umbilical artery sugar 75 mgm. Equilibration with the maternal sugar does not occur in the placenta but there is free uptake by the fetal tissues. Kettering-ham and Austin (1939) gave sugar to the mother intravenously in the second stage of labour. A rise in the sugar of both maternal and fetal bloods occurred returning to normal in four hours in the mother and in two hours in the fetus, suggesting active tissue-utilisation.

The placenta contains glycogen (Bernard, 1859) which appears at the 16th day in the rabbit (Pflüger, 1904), reaches a maximum at the 22nd day and then disappears toward term, coincident with a corresponding rise in fetal liver glycogen (Lochhead and Cramer, 1908). In man the peak is at the second month (Eufinger, 1932). Lochhead and Cramer (1908) showed it was difficult to alter the concentration of the placental glycogen experimentally. Huggett (1929) confirmed this and demonstrated it was only diminished by gross toxic katabolic

changes in the mother and that it was independent of the maternal blood sugar fluctuations. It appears to be a store of glycogen earmarked for the fetus, within the decidual tissue. Corey (1935) confirmed this in the rat. Endeavours have been made to study its metabolism by three main techniques: 1, autolysis of placental breis; 2, placental transfusion with sugars; 3, the Warburg technique.

The subject is reviewed by Needham (1931) but it is by no means in a satisfactory position because of difficulty of technique, differences in results obtained by different observers, differences in interpretation of results which do not always appear to be entirely objective. The following points appear to be established:

1. The placenta contains an amylase capable of converting glycogen to glucose (Lochhead and Cramer, 1908; Maeda, 1923).

2. The glycogen in aerobic autolysis of the placenta yields glucose (Davy and Huggett, 1934).

3. There is practically no production in vivo of lactic acid by either the placenta or embryo as shown by lactic acid estimations in the arterial and venous bloods of the uterine and umbilical vessels (Wind and von Oettingen, 1928).

4. Autoglycolysis of the embryo or placenta forms lactic acid anaerobically (Dickens and Greville, 1932).

5. All embryonic and placental tissues anaerobically form lactic acid from added glucose but only the placenta of man, the cat and the ferret and certain fetal membranes anaerobically form lactic acid from added fructose (Dickens and Greville, 1932).

There is also the possibility that perfusion of the placenta with glucose may form glycogen (Felix and von Oettingen, 1924).

The fetal liver glycogen in contrast to the placental glycogen is much more mobile, increasing in concentration with age in the rabbit (Lochhead and Cramer, 1908), the rat (Szendi, 1936) and man (Eufinger, 1932) and with fluctuations in the maternal blood sugar (Stuart and Higgins, 1935). The latter observers find it rises and falls more rapidly than the maternal liver glycogen. The observations of Lawrence (1940) and Ketteringham and Austin (1939) have been described above. Aron (1924) suggests the rise in fetal liver glycogen coincides with the development of the pancreas. Lund (1932), by special technique, has recorded that embryonic glycogen has a specific rotatory power less than adult liver glycogen and says it is specifically different.

Szendi (1936) says the lungs are relatively rich in glycogen in early

fetal life, before hepatic activity is maximal. The metabolism of glycogen in the fetal muscles is practically unstudied; Szendi regards it as a separate store.

Insulin (in regard to carbohydrate metabolism) appears not to pass across the placenta (Schlossmann, 1931; Snyder and Hoskins, 1928) either way. The ungulate fetus is relatively resistant to insulin compared with other types, and fetal lamb plasma if injected into the fetal rabbit confers resistance to insulin (Passmore and Schlossmann, 1938). Four hundred and fifteen units per kilogram gave no fall in blood sugar in the sheep but caused a big rise in blood lactic acid. The subject is reviewed by Schlossmann (1932a).

Lipin metabolism in placenta and fetus. Fat has been known to exist in the placenta for some time, the evidence for its presence being mainly histological. Watanabe (1923) showed that all the ether soluble materials in the human placenta increased up to the 4th month after which there was a progressive decrease till term. Kreidl and Donath (1910) had looked unsuccessfully for a lipase in the guinea-pig placenta which however was found in the human placenta by Anselmino and Hoffmann (1929) and also was found to have its maximum activity at the 4th month, possibly a coincidence with Watanabe's date given above. Hoffbauer (1903, 1905) and later Gage and Gage (1909) both showed that stained fat administered to the mother traversed the placenta but the dye was inhibited from passing and they inferred that the passage of fat was not by diffusion alone. Slemons (1919) and also Slemons and Stander (1923) showed fetal blood fat was uniformly less than the maternal blood fat. Oshima (1906) had shown this difference for the chylomicrons also and that the fetal chylomicrons (identified conclusively with fat particles by Elkes, Frazer and Stewart only in 1939) were unaffected by an increase in natural dietary fat sufficient to raise the maternal blood fat. Ahlfeld (1877) had obtained a similar result by giving bacon fat to the pig. Several observers showed that special types of fat traversed the placenta unaltered. These included Bickenbach and Rupp (1931) (fed labelled fat and cod liver oil to rabbit; detected unaltered in the fetal blood), Sinclair (1933) (cod liver oil in rats), McConnell and Sinclair (1937) (trielaidin in the rat appearing in the phospholipins of the fetal liver and fat deposits of the embryo). Sinclair (1933) concluded that there was no evidence in the rodent (whose placental membrane most resembles the human) of how the fats passed through the placenta, whether with hydrolysis or not. Needham (p. 1479) had pointed out that despite the lipase there was no evidence of forma-

tion of glycerol and fatty acids or of fat resynthesis in the placenta. Boyd (1935) estimated the neutral fat, phospholipin, free cholesterol and cholesterol esters in the placenta and fetus (rabbit) and concluded that increased phospholipin in the fetus was associated with great activity of the placenta and this was due to active and not passive transfer of the lipoids from mother to fetus. Boyd and Wilson (1935) showed that the human fetus at birth absorbs 30 grams of phospholipin and 7 grams of free cholesterol in 24 hours and that even if the umbilical cord were clamped the fat in the umbilical vein on the placental side increased showing, they inferred, active transfer of fat by the placenta. Sinclair (1933) had estimated iodine values in fetus and food fat. Thiemich (1905) had given coconut oil (I.V. 8.) to dogs in 1905 and showed that while it transfused it did not lower the fetal depot fat I.V. but did lower that of the maternal depots. Wesson (1926) found a similar result with the bromine value in rats and inferred the fetus synthesised fat from carbohydrate. Miura (1937), however, contradicted these results and confirmed Sinclair's iodine values, using rats and giving linseed oil, coconut oil and elaidic acid, which last appeared in the fetal phosphatids. Effkemann (1936) with infants at birth showed they absorb in 24 hours 33 grams of lecithin, 7.5 grams free cholesterol (essentially the same as Boyd and Wilson), 3 grams of fatty acid and 8.5 grams of cholesterol esters. The cholesterol esters are mainly carried by the serum, the free cholesterol and phospholipin by the cells. He inferred that the maternal diet can alter the fetal depot fat, and that preformed fat compounds are used by the fetus. Chaikoff and Robinson (1933) have shown that pregnant rats on a low fat diet (with or without an excess of carbohydrate or protein) have fetal fat stores of 72 to 74 iodine value. But if the dietary fat was much increased then the fetal I.V. ranged from 61 to 103 (maternal depot I.V. 36–145) and corresponded to the I.V. of the dietary fat. In other words, carbohydrate can be synthesised to fat of I.V. 72–74 in the fetus but this value can be altered if excess fat is given. Imrie and Graham (1920) studied the guinea-pig fetal liver fat. They found that its concentration rose steadily during intrauterine life giving pronounced fatty infiltration but there was a sudden sharp drop within 40 hours of birth (suggesting an acute birth change in metabolism). Its iodine value fell steadily from 120 to 110 but was always above the adult tissue I.V. of 85 (adult liver I.V. equals 120). These high values (120–110) suggested the fetal liver fat came directly from the adult liver unaltered or that the fetal liver had high desaturation power. They now

starved the pregnant guinea pig and gave phlorhizin. This produced double the fatty infiltration in the fetal liver but with fat of a low iodine value. This fresh fat they inferred as coming from the tissues or being synthesised from carbohydrate.

Some work has been performed on cholesterol metabolism. Boyd (1935) estimated the cholesterol in the placenta and fetus of the rabbit and showed the free sterol increased steadily in the placenta but in the fetus it rose first rapidly, then slowly and again steeply in the last ten days; cholesterol esters however rose in the first half and fell in the second half of pregnancy. With Wilson (1935) he estimated the human free cholesterol absorption per day, results confirmed later by Effkemann (1936). Chauffard, Laroche and Grigaut (1918, 1920) showed the human fetal suprarenal had a steadily rising percentage and absolute weight of free cholesterol with age; Sato (1937) demonstrated that it rises in the maternal blood and falls in the umbilical artery and vein throughout pregnancy and believed the fetus synthesised it. Rosenbloom (1935) with the full term infant estimated 223 mgm. cholesterol in the mother's blood and 120 mgm. in the umbilical blood and was undecided between fetal synthesising or fetal storage with progressive age.

Protein metabolism in placenta and fetus. Unlike carbohydrate there are no obvious protein stores, but in the last part of pregnancy the placenta gradually wastes. The products of the autolysis are unknown nor is there any knowledge of their fate. They are, however, formed at the time of maximal fetal protein synthesis and form an obvious foodstuff for the fetus.

Legrand (1936) states there are polypeptides in the maternal blood which in pregnancy are replaced by smaller molecule peptides which can diffuse through the placenta to be utilised by the fetus. This gives some support to the older view that fetal protein is synthesised from the albumoses. The results need confirmation however by an alternative method.

The circulating amino-acid content (whole blood and serum) is invariably higher in the fetus than in the mother. The data are reviewed by Slemons (1919), Needham (p. 1516) and Schlossmann (1932a, b). Despite these figures Schlossmann argues that amino-acids diffuse from the mother to the fetus. He states the fetal level is maintained artificially high by adsorption on to the red cells and colloids, quoting Plass and Matthew (1925) in support. It is doubtful, however, if the problem is so simple of solution. Luck and Engle (1929) showed that

the experimental injection of amino-acid into the mother speedily raises the fetal tissue content.

Protein deposition is maximal in the last quarter of pregnancy. There appears to be little relation between the composition of the proteins deposited in the fetus and that of the maternal proteins. The fetal creatine rises steadily throughout pregnancy (Beker, 1913; Hunter, 1922). On the other hand the nucleo-protein decreases with intrauterine age (Masing, 1911). Goldstein and Milgrom (1935) state cathepsin catalyses the protein deposition. It exists in two forms, activated and unactivated and the synthetic action is proportional to the excess of the former over the latter. They demonstrated that synthesis occurs at a slow rate in the human placenta but faster in rodents. They found protein synthesis both in the fetus and in the maternal tissues—not a wholly unexpected finding.

Slonaker (1938) has recently fed six generations of rats on diets containing constant protein rations between 10 and 26 per cent (man normally eats 13 per cent). He found high protein decreased birth weight but increased the post-natal rate of growth: both results, however, were less pronounced in successive generations.

Wilkerson and Gortner (1932) from analyses of fetal pigs for their protein content have decided that the nitrogenous deposition is fixed and governed by nature and not by the maternal nutrition but it is however helped by selective placental absorption.

Mineral metabolism in the placenta and fetus. Recent reviews covering the analyses and mineral deposition have been made by Swanson and Iob (1939) and Garry and Stiven (1936) which include the recent work of Givens and Macey (1933) in the human fetus. The following general points emerge. The rate of deposition varies for different minerals, sodium being deposited fastest in the beginning and middle of pregnancy, chloride in the beginning, potassium together with calcium and phosphorus fastest toward the end of pregnancy. Ramage (1929) has devised a spectrographic method of detaching and estimating small quantities of metals. He with Sheldon and Sheldon (1933) has shown iron is stored in increasing concentration in the first six months and in the remainder of pregnancy by the total fetal weight increasing but the percentage of iron keeping constant (human), yielding an increase in the absolute weight of iron. The placental iron rises steadily but its concentration falls at full term (4.37 mgm. per cent at 7th month to 2.66 mgm. per cent at term—Wagner, 1921). Hilgenburg (1930) showed prolonged gestation still further lowers the placental iron. Mackey's

(1931) study of infant anemias conclusively proved that anemic mothers deliver themselves of babies with normal hemoglobin percentage, which however very soon develop severe hypochromic anemia, but intensive iron treatment during pregnancy prevents the onset of the anemia in the infant after birth, suggesting the fetal iron stores were low though the hemoglobin was normal. Iob and Swanson (1938) did show that the bone stores of iron were very constant but in the fetal liver there was increasing percentage. Fetzer as long ago as 1913 showed iron deficiency in the diet of the pregnant rat depleted the iron content of the fetus, often causing abortions. The assimilation is altered by accompanying food (Vahlteich, Rose and Macleod, 1936) being depressed if accompanied by calcium carbonate or other earths (Kleitzen and Kingdon, 1936). There is practically no knowledge as to the form in which iron exists in the placenta or fetus. In the chick, however, MacFarlane and Milne (1934) have found a rising percentage of the total iron from 10 per cent at the 11th day to 50 or 60 per cent at the 21st day is non-hematin iron; this presumably has come from hemoglobin katabolism since Sendju (1927) has shown that bile production is biggest after the 16th day.

Copper has been studied by MacFarlane and Milne (1934) (chick), Wilkerson (1934) (pig) and Ramage, Sheldon and Sheldon (1933) (human and goat fetuses). In the human liver the copper concentration increases steadily in percentage and, unlike iron, in the later months its percentage goes on rising concurrently with the increase in weight of the liver; at term its concentration is double that in the adult liver. In the pig the copper kept constant throughout in concentration; with the chick it rises to a maximum concentration in early life and then keeps constant. MacFarlane and Milne regard the steadily rising percentage (compared with the constancy in the pig and chick embryos) as a measure of the free diffusion of copper across the hemo-chorial placenta of the human compared with the limitation in quantity imposed by the content of the original egg and in the pig by the epitheliochorial placenta. Incidentally vitellin contains copper. There appears to be little experimental work on placental transfusion of iron or copper apart from the quantitative observational papers above. Schlossmann (1932a) regarded the mode of passage as being transfusion. Garry and Stiven (1936) have reviewed the fetal demands with reference to the maternal diet.

Manganese occurs in traces in the human fetal liver (Ramage, Sheldon and Sheldon) and it has been estimated in the calf by Gruzewska

and Roussel (1937) but no one has obtained evidence as to its form or function, beyond that it does not accumulate like iron.

Calcium is present in the human placenta and increases in absolute weight toward term but decreases in its dry weight percentage (Wehefritz, 1925). Needham discusses its presence as calcium oxalate crystals in the embryotrophe and in the hippomanes-calcified masses floating in amniotic fluid and containing glycogen of unknown significance (p. 1458). The fetal blood calcium and inorganic phosphorus vary with but are always higher than the maternal blood values. Recent analyses have been made by Mull (1936), by Bang (1937) and by Sato (1938). There is considerable evidence that the fetus makes definite demands on the maternal exogenous metabolism and if the dietary supply is defective, then upon her endogenous calcium metabolism. Dibbelt (1910) and later Zuntz in 1919 decided that in dogs and rats low calcium diets caused no deficiency of calcium in the embryo. Sontag, Munson and Hoff (1936) however found in carefully controlled experiments that diets deficient in calcium, phosphorus and vitamin D formed rat embryos short of calcium and phosphorus (gravimetrically and radiologically). On adding therapeutic doses of vitamin D the calcium and phosphorus were raised to normal levels in the litters but toxic doses of vitamin D markedly lowered the phosphorus content. It is common clinical knowledge that after pregnancy women's teeth show signs of calcium deficiency and in the absence of vitamin D osteomalacia develops in the bones. Sherman and Macleod (1925) showed in rats that pregnancy lowers the calcium and phosphorus content of the mother rat. Toverud and Toverud (1929a) showed that commonly human pregnancies are associated with negative calcium-phosphorus balances, rectifiable however by calcium administration. In a later paper they showed in dogs that this negative balance led to histological and radiological loss of calcium and if suckled while the maternal diet was still low in calcium, rickets developed accompanied by low serum calcium and phosphorus. Booker and Hansemann (1931) showed the human fetus ossifies its bones efficiently and relatively independently of the maternal intake; in fact, was parasitic on the mother. Garry and Stiven (1936) reviewing the position confirm the League of Nations report on calcium and phosphorus, and after giving clinical and experimental instances showing the demands on the maternal metabolism and diet recommend that in the last three months the calcium and phosphorus be raised from 0.68 and 1.23 grams respectively to 1 or 2 grams and 2 grams each per day. Apart from the papers of Toverud

and Toverud (1929a) and Sontag, Munson and Hoff (1936) there is little work on the effect of vitamin D in calcium-phosphorus metabolism and no evidence on the effects of parathyroid in the fetus. Burns and Henderson (1934, 1935) have shown that bone laid down in late fetal life has a low water content and a high carbon dioxide concentration and a high degree of calcification. They feel the fetal blood composition is not the most important factor controlling bone formation.

Toverud and Toverud (1929b) found magnesium injections in part rectified calcium deficiency. Further information is obtainable from Needham (1931) and Duckworth (1939).

Numerous other metals have been detected by spectroscopic methods but their rôle is not clear. (Sheldon and Ramage, 1931; Rusoff and Gaddum, 1918; Iob and Swanson, 1934.)

Shohl (1923) in his review on the maintenance of acid-base balance has pointed out the human fetus in the last two months takes daily from its mother 230 cc. of 0.1 N base and 145 cc. of 0.1 N acid radicle, a daily excess of 85 cc. of 0.1 N basic radicle.

Vitamins and the embryo. Maxwell reviewed the subject of vitamins and pregnancy in 1932.

Vitamin A. The following points seemed to be established in regard to vitamin A:

1. The placenta impedes its rapid transmission as shown by Wendt (1936) in the human and Dann (1934) in the rat.

2. It is stored in the fetal liver in high concentration especially in the early months. (Wendt, 1936; Neuweiler, 1936a and Gaehtgens, 1937a.) Gaehtgens (1937b) found a tendency to store in the placenta and Dann found increasing the fat content of the diet increased the amount which passed across the placenta. It probably accumulates wherever fat is to be found.

3. Deficiency lowers the total litter weight in rats (Korenchewsky and Carr, 1923), possibly of individual fetuses (Abels, 1922); it also produces fetal abnormalities if gross (Hale, 1933; Hughes, 1934) and fetal death which Mason (1935) shows to be due to maternal abnormalities and curable by cod liver oil. This confirms Evans (1928) who said absence of vitamin A impaired the maternal reproductive system so that fertilisation and implantation failed. This contrasts with vitamin E deficiency (see below). Green, Pindar, Davis and Mellanby (1931) showed that in pregnant women A-deficiency results in septic conditions, which can also be reproduced in rats: these effects may influence the fetus. Vitamin A concentrates increase birth weight. (Massachusetts 1938.)

Vitamin D. This appears to store particularly well in the human placenta (Guggisberg, 1929) and to be present in the fetal liver and in particularly high concentration in the premature infant's liver (Hess and Weinstock, 1938). Abels (1922) thought it occurred with vitamin A in excess in summer foodstuffs and influenced the birth weights where the mother was delivered in August. Abel (1931) showed it prevented rickets when given to pregnant animals and hardened fetal skull bones. Finola (1937) and Foa (1937) showed either the vitamin or sunlight increased the calcium-phosphorus deposition, thickened the skull, but did not increase phosphatase.

Vitamin E. Like the other fat soluble vitamins, it is held up at the placenta (Mason and Bryan, 1938). Evans, Burr and Althausen (1927) showed it was normally present in the newborn rat but that its absence allows healthy implantation of fertilised ova but there the normal pregnancy terminates. Urner (1931) demonstrated rarefaction of the fetal hematopoietic and mesenchymal tissues. Barrie (1939) has suggested its deficiency besides producing these fetal symptoms also produces maternal pregnancy toxemia (fatty infiltration of the liver, etc.). This would of course affect the nutrition of the fetus adversely.

Vitamin B. With rats on a minimal diet of the complex, abortion or fetal resorption is common and even if they come to birth they often die of polyneuritis (Moore and Brodie, 1927). These authors also report a case of gestational beri-beri with B 1 deficiency such as occurs in India and Portland, U. S. A. Maxwell (1932) shows latent pellagra due to lack of the pellagra-preventing factor (denoted by him in 1932 as B 2) may be unmasked by pregnancy. Neuweiler (1937) has shown that flavin has a wide distribution in the rat's fetal liver and placenta and there is no relation between age and organ content.

Vitamin C. A considerable amount of work has been performed since the discovery of ascorbic acid and its method of estimation. A full review of its position in pregnancy has been made by Garry and Stiven (1936). The most important points that emerge are the following.

1. Shortage in the maternal diet may cause premature births (Elmby and Becker-Christensen, 1938) and with experimental guinea pigs a deficiency in the early stages of pregnancy causes abortion and maternal scurvy but if deficient in late pregnancy the mother has no lesions of scurvy but the fetus has such lesions; however, the mother after delivery of the scorbutic young develops the symptoms of scurvy (Mouriquand, 1935, and Mouriquand, Gillet and Coeur, 1935).

2. The vitamin C content of the fetal blood is higher than that of the

mother (Wahren and Rundqvist, 1937) and both values are raised by orange juice (Manahan and Eastman, 1938) (Fleming and Sanford, 1939). Neuweiler (1935) showed there is twice as much in the blood of the umbilical vein as in that of the umbilical artery and that the fetal (human) suprarenal cortex has a higher concentration than the adult cortex. Giroud (1936a) believes synthesis of ascorbic acid occurs in animal embryos and also (1936b) in the human fetuses. Mouriquand, Coeur and Viennois (1936) feel, however, that the fetus does not synthesise vitamin C—a surprising possibility in a fetus in view of the scorbutic lesions of their earlier paper (1935b) and the absence of the power in adult animals. Neuweiler (1936b) thought that ascorbic acid is stored by the placenta and decidua and the quantity varied with the maternal diet but Muller (1939) from examinations of the cow, pig, horse and man is against storage or synthesis of ascorbic acid by the fetus.

It seems that the water soluble vitamins traverse the placenta easily in contrast to the fat soluble vitamins. On the other hand the fat soluble vitamins so far as the evidence goes seem to store in fatty organs especially the fetal liver whereas the water soluble vitamins appear to have little power of storage apart from the known peculiarity of the suprarenal cortex for concentrating ascorbic acid.

The intermediate metabolism of the fetus. The changes undergone by the foodstuffs have been discussed in connection with their separate metabolisms. Needham covers the work up to 1931 and since then very little has been done. Numerous enzymes have been identified but their part in the complete story is not clear and their function is not always obvious. For example, although lipase has been found in the placenta, there is no evidence that the passage of fats across it is dependent upon the presence of the enzyme. Again enzymes have been shown to develop in the fetus at different dates but the tests by which they have been identified do not show they are acting in the fetus as in the adult: as for example the presence of digestive enzymes in the fetal intestinal tract. The same generalization applies to hormones. There is a lack—owing to the difficulty of the technique—of experiments designed to show that an agent whose presence has been demonstrated by some *in vitro* or other external test, is in fact performing a physiological function in the fetus or placenta. In this connection it is of interest to note Sax and Leibson (1937) showed that after thyroidectomy fertilisation and implantation of the ovum took place followed later by its death. Development however did progress normally if it was trans-

planted to the uterus of an unthyroidectomised female. Again adrenalin has been found in most fetal suprarenals but never in that of man. Newton (1938) has reviewed the hormones of the placenta. They are confined almost entirely to the sex and related pituitary hormones. He shows there are stores of these endocrines and adduces evidence pointing to the placenta as beng a ductless gland which secretes hormones akin in activity to the known "sex" hormones.

This aspect, however, leads to the fundamental underlying development. The whole question of the nutrition of the fetus is bound up in the basic problems of the factors causing the fertilised ovum to grow, causing differentiation at different ages, causing species differences in different animals, causing development of organs before the demands of functional activity are presented. In the case of the placenta we have the interesting complication of senescent changes before the end of uterine life, a reverse of the developmental changes in earlier uterine life. The very close chemical relationships between the structure of enzymes of widely different action, such as progesterone, oesterone and androsterone, suggest that they probably are derived from an inactive precursor capable of splitting off and forming one or the other or several similar active bodies of differing action. It is not therefore surprising to find these endocrines in an autolysing organ such as the placenta. It would be of interest to know if similar products appear in autolysis elsewhere.

The metabolic errors studied by Garrod (1923) and the work of Penrose (1935, 1937) on genetic factors influencing the fetal and post-natal nutrition together with the recent work on viruses and on the related nucleoproteins indicates lines of approach to the basic problems which so far have only been touched on with regard to mammalian development.

There are certain peculiarities existent distinguishing man (and the Primata) from the other mammals in fetal and post-natal life. Rubner (1908) indicated the slow rate of growth as one. It has been shown that the high sugar content of the human milk was of the order associated with muscular activity at birth (foal, kid), which is absent in the infant, however.

In the mammal we can, taking birth weight as the principal measure of nutritional fitness, obtain the best results by breeding from good stock, by breeding from mothers of adult weight, by breeding from mothers on a high plane of nutrition. Where moderately undernourished, however, the fetal nutrition is not impaired but two things

will occur, first the maternal nutrition will be lowered still more, as shown by her condition in the puerperium (this raises the interesting problem of why it is not exhibited during pregnancy and whether the fetus exerts a controlling protecting influence on her metabolism). The second effect appears to be that although the fetal functioning metabolism is effective, the reserves are deficient, especially in regard to iron. This shortage can show itself by hypochromic anemia and postnatal malnutrition requiring extra nourishment. If the mother is grossly undernourished then does the fetus show signs before birth of malnutrition.

The fetus increases its weight mainly in the last quarter or fifth of pregnancy. It draws for minerals, for proteins and for carbohydrates on the maternal supplies, though it is not known how much comes from the mother's diet, her tissues or the autolysing placenta. It appears to build up its fat from that which it receives or to synthesise it from the carbohydrate traversing the placenta. The mother's diet if adequate does spare her tissues, but needs to be increased at this period over the non-pregnant levels of health.

REFERENCES

ABEL, K. Ztschr. Ernährung **1**: 266, 1931.

ABELS, H. Klin. Wchnschr. **1**: 1785, 1922.

ACOSTA-SISON, H. AND J. CALANG. J. Philippine Is. Med. Assoc. **11**: 272, 1931.

AHLFELD, J. F. Zentralbl. Gynäk. **15**: 265, 1877.

ANSELMINO, K. J. AND F. HOFFMAN. Arch. Gynäk. **139**: 202, 1929.

ARON, M. Arch. Internat. Physiol. **22**: 273, 1924.

BANG, T. Acta paediat. japon. **43**: 408, 1937.

BARRIE, M. M. O. J. Obstet. Gynaec. **46**: 49, 1939.

BEKER, J. C. Ztschr. physiol. Chemie **87**: 21, 1913.

BERNARD, C. J. Physiol. de l'homme **2**: 336, 1859.

BICKENBACH, W. AND H. RUPP. Klin. Wchnschr. **10**: 63, 1931.

BOOHER, L. E. AND G. H. HANSEMANN. J. Biol. Chem. **94**: 195, 1931.

BOYD, E. M. Biochem. J. **29**: 985, 1935.

BOYD, E. M. and K. M. WILSON. J. Clin. Investigation **14**: 7, 1935.

BRODY, S. In Growth by W. J. ROBBINS and others. New Haven, 1928.

BURNS, C. M. AND N. HENDERSON. J. Physiol. **82**: 7, 1934.
J. Physiol. **83**: 46, 1935.

CHAIKOFF, I. L. AND A. ROBINSON. J. Biol. Chem. **100**: 13, 1933.

CHAUFFARD, A., G. LAROCHE AND A. GRIGAUT. C. R. Soc. Biol. Paris **81**: 87, 1918.
Ann. Méd. **8**: 149, 1920.

COREY, E. L. Am. J. Physiol. **113**: 450, 1935.

CUNNINGHAM, R. S. Am. J. Physiol. **53**: 439, 1920.

DANIELLI, J. F. J. Physiol. **98**: 19 P, 1940.

DANN, W. J. Biochem. J. **28**: 634, 1934.

DAVY, A. AND A. ST. G. HUGGETT. J. Physiol. **81:** 183, 1934.
DIBBELT, W. Beitr. path. Anat. **48:** 147, 1910.
DICKENS, F. AND G. D. GREVILLE. Biochem. J. **26:** 1251, 1932.
DIETRICH, H. Article in HALBAN AND SEITZ: Biologie und Pathologie des Weibes. Vol. VI. Berlin, 1925.
DUCKWORTH, J. Nutrit. Abstr. and Rev. **8:** 856, 1939.
ECKLES, C. H. Missouri Agric. Expt. Sta. Res. Bull. no. 35, 1919.
EFFKEMANN, G. Arch. Gynäk. **162:** 148, 1936.
ELKES, J. J., A. C. FRAZER AND H. C. STEWART. J. Physiol. **95:** 68, 1939.
ELLIOTT, R. H., F. G. HALL AND A. ST. G. HUGGETT. J. Physiol. **82:** 160, 1934.
ELMBY, A. AND P. BECKER-CHRISTENSEN. Ugeskr. Laeg. **100:** 1047, 1938.
EUFINGER, H. Monatschr. Geburtsh. Gynäk. **92:** 272, 1932.
EVANS, H. M. J. Biol. Chem. **77:** 651, 1928.
EVANS, H. M., G. O. BURR AND T. L. ALTHAUSEN. Memoirs Univ. California, vol. 8, 1927.
FELIX, K. AND K. VON OETTINGEN. Monatschr. Geburtsh. Gynäk. **67:** 41, 1924.
FETZER, M. Ztschr. Geburtsh. Gynäk. **74:** 542, 1913.
FINOLA, G. C., R. A. TRUMP AND M. GRIMSON. Am. J. Obstet. and Gynec. **34:** 955, 1937.
FLEMING, A. W. AND H. N. SANFORD. J. Pediat. **13:** 314, 1938.
FOA, P. Arch. Fisiol. **37:** 198, 1937.
GAEHTGENS, G. Klin. Wchnschr. **16:** 1073, 1937a.
Klin. Wchnschr. **16:** 1075, 1937b.
GAGE, S. AND S. GAGE. Anat. Rec. **3:** 203, 1909.
GAMGEE, A. Brit. and Foreign Med-Chir. Rev. **33:** 180, 1864.
GARROD, A. E. Inborn errors of metabolism. London, 1923.
GARRY, R. C. AND D. STIVEN. Nutrit. Abstr. and Rev. **5:** 855, 1936.
GIROUD, A. AND OTHERS. C. R. Soc. Biol. Paris **121:** 1062, 1936a.
C. R. Soc. Biol. Paris **123:** 1038, 1936b.
GIVENS, M. H. AND I. G. MACEY. J. Biol. Chem. **102:** 7, 1933.
GOLDSTEIN, B. AND E. J. MILGROM. Ukraine Biochem. J. **8:** 139, 1935. (Quoted from Nutrit. Abstr., Vol. V.)
GREEN, H. N., D. PINDAR, G. DAVIS AND E. MELLANBY. Brit. Med. J. **2:** 595, 1931.
GREENE, C. V. J. Exper. Zool. **59:** 247, 1931.
GROSSER, O. In Halban and Seitz' Biol. u. Path. d. Weibes **6:** 1, Berlin, 1925.
GRUZEWSKA, Z. AND G. ROUSSEL. C. R. Soc. Biol. Paris **126:** 965, 1937.
GUGGISBERG, H. Deutsch. Med. Wchnschr. **53:** 1953, 1929.
GUTHMAN, H. AND L. BOHME. Arch. Gynäk. **92:** 272, 1932.
HALE, F. Heredity **24:** 105, 1933.
HAMMOND, J. Growth and development of mutton qualities in the sheep. Edinburgh, 1932.
HARRIS, H. A. J. Anat. **64:** 1, 1929.
Bone growth in health and disease. Oxford, 1933.
HAYASHI, S. Biochem. Ztschr. **196:** 323, 1928.
HESS, A. F. AND M. WEINSTOCK. Am. J. Dis. Child. **36:** 966, 1928.
HESS, J. H. AND I. MCK. CHAMBERLAIN. Am. J. Dis. Child. **34:** 571, 1927.
HILGENBURG, F. C. Ztschr. Geburtsh. Gynäk. **98:** 291, 1930.

HOFBAUER, J. Ztschr. Physiol. **39:** 458, 1903.
Biol. d. Mensch. Plazenta, Wien, 1905.
HOGAN, A. G. Growth by W. J. ROBBINS and others. New Haven, 1928.
HUGGETT, A. ST. G. Unpublished observation, 1928.
J. Physiol. **67:** 360, 1929.
HUGHES, E. H. J. Am. Vet. Med. Assoc. **84:** 936, 1934.
HUNTER, A. Physiol. Rev. **2:** 590, 1922.
ILLINGWORTH, R. S. Arch. Dis. Child. **14:** 121, 1939.
IMRIE, C. G. AND S. G. GRAHAM. J. Biol. Chem. **44:** 243, 1920.
IOB, V. AND W. W. SWANSON. Am. J. Dis. Child. **47:** 302, 1934.
J. Biol. Chem. **124:** 263, 1938.
JONEN, P. Arch. Kinderheilk. **98:** 32, 1932.
JONES, F. WOOD. Personal communication, 1939.
KETTERINGHAM, R. C. AND B. R. AUSTIN. Am. J. Obstet. Gynaec. **37:** 1000, 1939.
KLEITZEN, S. W. AND C. KINGDON. J. Nutrition **11:** Suppl. p. 16, 1936.
KORENCHEVSKY, V. AND M. CARR. Biochem. J. **17:** 597, 1923.
KREIDL, A. AND H. DONATH. Zentralbl. Physiol. **24:** 2, 1910.
LAWRENCE, R. D. Quart. J. Med. **22:** 191, 1929.
Personal communication, 1940.
LEGRAND, G. Brux. Méd. **16:** 1131, 1936.
LOCHHEAD, J. AND W. CRAMER. Proc. Roy. Soc. B. **80:** 265, 1908.
LOESER, A. Klin. Wchnschr. **13:** 587, 1927.
LUCK, J. M. AND E. T. ENGLE. Am. J. Physiol. **88:** 230, 1929.
LUND, H. C. R. Soc. Biol., Paris **110:** 1121, 1932.
MCCONNELL, K. P. AND R. G. SINCLAIR. J. Biol. Chem. **118:** 123, 1937.
MCDOWALL, E. C., W. H. GATES AND C. G. MCDOWALL. J. Gen. Physiol. **13:** 529, 1930.
MACFARLANE, W. D. Biochem. J. **26:** 1061, 1932.
MACFARLANE, W. D. AND H. J. MILNE. J. Biol. Chem. **107:** 309, 1934.
MACKEY, H. M. M. Med. Res. Counc. Spec. Rept. Series no. 157, 1931.
MAEDA, K. Biochem. Ztschr. **143:** 347, 1923.
MANAHAN, C. P. AND N. J. EASTMAN. Bull. Johns Hopkins Hosp. **62:** 478, 1938.
MARSHAK, A. J. Exper. Zool. **72:** 497, 1936.
MARSHALL, F. H. A. Physiology of reproduction. London, 1922.
MASING, E. Ztschr. physiol. Chem. **75:** 135, 1911.
MASON, J. H., T. DALLING AND W. S. GORDON. J. Path. Bact. **33:** 783, 1930.
MASON, K. E. Am. J. Anat. **57:** 303, 1935.
MASON, K. E. AND W. L. BRYAN. Anat. Rec. **70:** Suppl. no. 3, 55, 1938.
Massachusetts Agric. Expt. Stat. Bull. no. 347. Nutrit. Abstr. **8:** 551, 1938.
MAXWELL, J. P. J. Obstet. Gynaec. **39:** 764, 1932.
MELLANBY, J. J. Physiol. **64:** 331 and p. V, 1927.
MENDEL, L. B. AND A. L. DANIELS. J. Biol. Chem. **13:** 71, 1912.
MINOT, C. S. J. Physiol. **12:** 97, 1891.
The problem of age, senescence and growth. London, 1908.
MIURA, K. J. Biochem. **25:** 579, 1937.
MOORE, C. V. AND J. L. BRODIE. Am. J. Dis. Child. **34:** 53, 1927.
MOSSMAN, H. W. Am. J. Physiol. **37:** 433, 1926.
MOURIQUAND, G. Bull. Acad. Méd. Paris **114:** 199, 1935.

MOURIQUAND, G., R. GILLET AND A. COEUR. Pr. méd. **43:** 1578, 1935.
MOURIQUAND, G., A. COEUR AND P. VIENNOIS. C. R. Soc. Biol. Paris **121:** 1005, 1936.
MULL, J. W. J. Clin. Investigation **15:** 513, 1936.
MULLER, J. Klin. Wchnschr. **18:** 299, 1939.
NEEDHAM, J. Chemical embryology. Cambridge, 1931.
NEGELEIN, E. Biochem. Ztschr. **165:** 122, 1925. (In English in WARBURG, 1930.)
NEUWEILER, W. Klin. Wchnschr. **14:** 1040, 1935.
Ztschr. Vitaminforsch. **5:** 104, 1936a.
Arch. Gynäk. **162:** 384, 1936b.
Ztschr. physiol. Chem. **249:** 225, 1937.
NEWTON, W. H. Physiol. Rev. **18:** 419, 1938.
OSHIMA, T. Zentralbl. Physiol. **21:** 297, 1906.
PASSMORE, R. AND H. SCHLOSSMANN. J. Physiol. **92:** 459, 1938.
PATON, D. N. Lancet **2:** 21, 1903.
PENROSE, L. S. Lancet **2:** 192, 1935.
PENROSE, L. S. AND J. H. QUASTEL. Biochem. J. **31:** 266, 1937.
PFLÜGER, E. Pflüger's Arch. **102:** 305, 1904.
PLASS, E. D. AND C. W. MATTHEW. Bull. Johns Hopkins Hosp. **36:** 393, 1925.
PROCHOVNIK, L. Centralbl. f. Gynäk. Geburtsh. **13:** 577, 1899.
Therap. Monatschr. **15:** 446, 1901.
RAMAGE, H. Nature **123:** 601, 1929.
RAMAGE, H., J. H. SHELDON AND W. SHELDON. Proc. Roy. Soc. B **113:** 308, 1933.
RATNER, B., H. C. JACKSON AND H. L. GRUEHL. J. Immunol. **14:** 275, 1927.
REEB, B. Beitr. Geburtsh. Gynäk. **9:** 395, 1905.
ROBERTSON, T. B. The problem of age, growth and death. London, 1923.
ROSENBLOOM, D. Proc. Soc. Exper. Biol. Med. **32:** 908, 1935.
RUBNER, M. Problem der Lebensdauer. Berlin, 1908.
RUSOFF, L. L. AND J. N. GADDUM. J. Nutrition **15:** 169, 1918.
SATO, Y. J. Chosen. Med. Assoc. **27:** 1937. (From Nutr. Abstr. **7:** Abstr. 471.)
J. Chosen. Med. Assoc. **28:** 44, 1938. (From Nutr. Abstr. vol. 8.)
SAX, M. G. AND R. G. LEIBSON. Bull. Biol. Med. exp. U.R.S.S. **4:** 496, 1937. (Nutrit. Abstr. **8:** 674.)
SCHLOSSMANN, H. Ztschr. ges. Exper. Med. **62:** 401, 1930.
Arch. exper. Path. und Pharmakol. **159:** 213, 1931.
Ergebn. Physiol. **34:** 741, 1932a.
Arch. exper. Path. und Pharmakol. **166:** 81, 1932b.
SCHMALHAUSEN, J. AND J. STEPANOVA. (Quoted in NEEDHAM, 1931, p. 441), 1926.
SHELDON, J. H. AND H. RAMAGE. Biochem. J. **25:** 1608, 1931.
SHERMAN, H. C. AND F. L. MACLEOD. J. Biol. Chem. **64:** 429, 1925.
SHOHL, A. T. Physiol. Rev. **3:** 509, 1923.
SINCLAIR, R. G. Am. J. Physiol. **103:** 73, 1933.
SLEMONS, J. M. Nutrition of the foetus. New Haven, 1919.
SLEMONS, J. M. AND H. S. STANDER. Bull. Johns Hopkins Hosp. **34:** 7, 1923.
SLONAKER, J. R. Am. J. Physiol. **123:** 526, 1938.
SNYDER, F. F. AND F. M. HOSKINS. Anat. Rec. **38:** 28, 1928.
SONTAG, L. W., P. MUNSON AND E. HOFF. Am. J. Dis. Child. **51:** 302, 1936.
STRAUSS, M. B. J. Clin. Investigation **12:** 345, 1933.

STUART, H. A. AND G. M. HIGGINS. Am. J. Physiol. **111**: 590, 1935.
SWANSON, W. W. AND V. IOB. Am. J. Obstet. Gynecol. **38**: 382, 1939.
SZENDI, B. Arch. Gynäk. **162**: 27, 1936.
THIEMICH, M. Jahrb. Kinderheilk. **61**: 174, 1905.
TOVERUD, K. Am. J. Dis. Child. **46**: 954, 1933.
TOVERUD, K. AND G. TOVERUD. Skand. Arch. Physiol. **55**: 281, 1929a.
Skand. Arch. Physiol. **55**: 282, 1929b.
URNER, J. A. Anat. Rec. **50**: 175, 1931.
VAHLTEICH, E. McC., M. S. ROSE AND G. MACLEOD. J. Nutrition **11**: 31, 1936.
VERZAR, F. AND McDOUGALL, E. J. Absorption from the intestine. London, 1936.
WAGNER, R. Ztschr. Kinderheilk. **27**: 251, 1921.
WAHREN, H. AND O. RUNDQVIST. Klin. Wchnschr. **16**: 1498, 1937.
WALTON, A. AND J. HAMMOND. Proc. Roy. Soc. B **125**: 311, 1938.
WARBURG, O. Metabolism of tumours. English ed. London, 1930.
WATANABE, H. J. Biochem. **2**: 369, 1923.
WEHEFRITZ, E. Arch. Gynäk. **127**: 106, 1925.
WENDT, H. Klin. Wchnschr. **15**: 222, 1936.
WESSON, L. Bull. Johns Hopkins Hosp. **38**: 237, 1926.
WILKERSON, V. A. J. Biol. Chem. **104**: 541, 1934.
WILKERSON, V. A. AND B. A. GORTNER. Am. J. Physiol. **102**: 153, 1932.
WIND, F. AND K. VON OETTINGEN. Biochem. Ztschr. **127**: 170, 1928.
Reproduced in English in WARBURG, 1930.
ZUNTZ, L. Arch. Gynäk. **110**: 244, 1919.
In OPPENHEIMER's Handbuch d. Biochemie d. Menschen u. d. Tiere. **7**: 132, 1924.

Considerable use has been made of the following books in regard to the comparative physiology and anatomy of the placenta and fetus:

HALBAN AND SEITZ. Biologie u. Pathologie des Weibes. Vol. VI, Berlin, 1925.
JENKINSON, J. W. Vertebrate embryology. Oxford, 1913.
MARSHALL, F. H. A. Physiology of reproduction. London, 1922.
NEEDHAM, J. Chemical embryology. Cambridge, 1931.

THE CIRCULATION OF THE BILE ACIDS IN CONNECTION WITH THEIR PRODUCTION, CONJUGATION AND EXCRETION

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The entero-hepatic circulation of the bile acids has been known for a considerable time. In 1863 Hoppe-Seyler found that very little of the bile acid present in the bile could be found in the feces and he concluded that it was resorbed from the intestines. In 1870 Schiff showed the choleretic effect of bile given by mouth or directly into the intestines and he attributed this effect to the circulation of the bile. Finally Tschernoff (1884) and Stadelmann (1896) proved the resorption of bile acids from the intestines and their resorption through the liver.

Since then the circulation of the bile acids has been studied and confirmed by numerous investigators. We now know that the bile salts are resorbed from the intestine, carried to the liver by the portal blood and again excreted with the bile. Only a small part of the bile acids is lost in each of these turns and a correspondingly small part is synthesised. If extra bile salts are supplied by intravenous injection they are almost immediately adsorbed by the surfaces of the blood-vessel system and very soon incorporated into the normal circulation. If given by mouth they also enter the circulation. Normally most of the bile acids in the bile are conjugated with glycine or taurine. If unconjugated acids are supplied they will be conjugated by the liver to a certain degree.

By the circulation of the bile acids these physiologically valuable substances are saved to a considerable extent. The same amount of bile acid may be used again and again for the resorption of fats, vitamins or other lipoids and in this way it may transport many times the amount of lipoid which it is able to keep in solution by coördination. In man the bile acids seem to pass the circulation about three times and in dogs about seven times. The significance of the bile acids for the lipoid resorption will not be discussed here.

In jaundice the normal bile circulation is blocked. If the icterus is due to damage of the liver parenchyma as in different kinds of hepatitis there is very little or no passage through the parenchyma cells to the bile capillaries. Usually the contact is better between the cells and the blood passing the liver. The inflamed liver can take up and deal with many of the substances carried to it by the portal and peripheral blood, but it has marked difficulty in absorbing several substances among which are the bile salts. For this reason these salts remain for a long time in the circulating blood if injected intravenously into hepatitic patients or animals.

In obstructive jaundice, finally, the normal bile circulation is also broken. But here the contact between the blood and the liver cells and between the liver cells and the bile capillaries is still unbroken, at least provided there is little or no secondary cell-damage. On the other hand the slight secretion pressure of the bile (in cases of low obstruction increased by the muscular pressure of the gall bladder) is enough to open the intercellular spaces between the parenchyma cells of the liver. In this way a new outflow is opened and bile can escape from the bile capillaries and reach the thoracic duct and the circulating blood via the lymph spaces and lymph vessels of the liver. Thus also in obstructive jaundice there is a kind of bile circulation. If bile salts are injected intravenously into patients or animals with obstructive jaundice the immediate disappearance is not so rapid as in normal circumstances, but the subsequent decrease in concentration is rapid as compared with that in hepatitis. This is owing to the continued capacity of the liver cells to take up the acids and to keep them circulating.

In practice (and especially in clinical cases) these two types of reaction, the hepatitic and the obstructive, are seldom quite clearly differentiated, as damage of the parenchyma usually causes an edema with some obstruction of the bile capillaries. In hepatitis, however, an opening of the lymph spaces seldom takes place unless an extensive destruction of liver cells occurs. Obstruction, on the other hand, usually causes some cell damage, sooner or later, by pressure or infection.

The literature concerning the circulation of the bile acids as also their physiology in general is rather overwhelming. Most of this literature up to 1937 is summarized in the excellent book by Sobotka.

Analytical Methods. Many of the studies involving analyses of the bile itself were at an early stage marked by a considerable reliability as the determinations here are easy to carry out and the methods were usually rather good. However, the numerous investigations based on

blood determinations are all of limited value, since most of the earlier methods for the determination of bile salts in the blood are very unspecific.

In the investigations by the author of the present paper and co-workers the analyses of the bile have been carried out according to previous well known methods evolved by Josephson and Jungner (1936). For the analysis of cholic acids a modification of the Gregory and Pascoe (1929) method has been used and for determinations of conjugated acids a modification of the method of Schmidt and Dart (1920/21). Concerning errors in the latter method see Doubilet and Colp (1936). The blood determinations are according to Josephson (1935) with the slight but significant modification described by Josephson and Larsson (1939). This method, like almost all of the previous ones, is nothing but a far-going modification of the old Pettenkofer (1844) test, which in its original form is not specific. However, we believe that we have proved the blood method as it is now carried out to be rather specific and with an average error of less than ± 10 per cent. It may be admitted that a small part of the color developed in the reaction may be the result of interfering non-specific substances, especially in the low normal values, but, as further emphasized by Josephson (1939b), their influence must be of very little importance. The main point of the method lies in a thorough-going isolation of the bile salts from other constituents of the blood. The determination itself depends on the well known colorimetric reaction with furfural and strong sulphuric acid which is read in the step-photometer of Pulfrich.[1] It must be clearly pointed out that by this method only cholic acid and its derivatives can be determined. No reliable method for the estimation of litho- or deoxycholic acids in blood has yet been published. Several modifications of the method mentioned above have been published recently (Abe, 1937; Lichtmann, 1938).

The Entero Hepatic Circulation. Resorption of the bile acids from the intestine. When the fatty acids and lipoids, combined with bile acids to soluble choleic acids (Tschernoff, 1884; Wieland and Sorge, 1916; Verzár and Kuthy, 1929) are resorbed from the intestines, the combination is again dissociated already in the mucosa cells. The fats are carried further, mainly by the lymph, but the bile acids have a tendency to stick to the intestinal walls, where they combine with new

[1] It is of special importance if one is to get good values with the method that the ethyl acetate used for the separation be pure and free from acetic acid. Unfortunately, the method is too time-consuming for routine clinical use.

fats (Verzár and Kuthy, 1929). Verzár and Kuthy (1930) found that if a bile salt solution was locked up in a piece of the small intestine a considerable part of the salts could soon be found in the wall itself, while the concentration in the fluid content decreased. The bile salts in the wall were thus assumed to act as fat transporters and in this way a small amount of salts may transport a considerable amount of fat through the mucosa even without taking part in the circulation (Riegel, O'Shea-Elsom and Ravdin, 1935).

To some extent, the bile salts themselves are also gradually carried further. When being resorbed, however, they do not follow the fats into the lymph but are transported directly to the liver by the portal blood. Thus, Josephson and Rydin (1936) always found a higher cholate concentration in the portal blood than in blood obtained by heart puncture. In rabbits and cats the concentration in blood from the portal vein was 2 to 5 mgm. per 100 ml. and in heart blood only 1 to 2 mgm. per 100 ml. As previously found by Greene, Aldrich and Rowntree (1928) this difference increases if a bile salt solution is injected into the intestine of the animal. On the other hand, Josephson and Rydin (1936) did not find any difference at all in animals having no bile in the intestine owing to a previous ligation of the common bile duct. These results were confirmed on dogs by Josephson and Kaunitz (1937) in an investigation where also the thoracic lymph was examined. In normal dogs this lymph never contained cholates in concentrations high enough to be determined even during extensive resorption of bile salts. The cholates in the portal blood of their dogs were markedly increased during bile resorption. The results have been confirmed by Jenke and Graff (1939) who found corresponding differences between blood of different origin, only with lower values throughout.

According to Tappeiner (1878), the resorption takes place chiefly in the ileum, no bile salts being resorbed in the duodenum, while only glycocholic acid in the jejunum. However, his results cannot be considered decisive, as in his experiments the intestinal mucosa was strongly irritated and bleeding (probably due to too strong alkalescense of the bile salt solutions) and the conditions were thus very unphysiological. However, his results have been confirmed by Frölicher (1935/36).

Sobotka (1937, p. 38) has made an attempt to calculate roughly the difference that might be expected between the bile salt concentration in portal and in peripherical blood, basing his results upon the bile salt excretion of bile fistula dogs according to Foster, Hooper and Whipple (1919) and found that this difference would be about 0.7 mgm. per 100

ml. of blood. He obtained the figure of the volume of blood passing the liver by calculating it to be proportional to the weight of this organ as compared with the body-weight. He also calculated the amount of bile acids produced per 24 hours and kilogram body-weight to be 0.115 gram, which is the endogenous production found by Whipple and Smith (1930) when the fistula bile was not given back by mouth to the dogs. However, Schwiegk (1932) has shown that the portal blood flow is increased by 100 to 200 per cent during resorption, and according to Whipple and Smith the bile acid excretion of fistula dogs is about 0.800 gram per kilogram body-weight when the fistula bile is re-fed to the dog which was assumed to be about the same as in normal circumstances. If these figures are used in Sobotka's formula, the difference between the bile acid content of portal blood and that of peripheral blood would be approximately about 2 mgm. per 100 ml. As the figures of Whipple and Smith (1930), confirmed by Schmidt, Beazell, Berman, Ivy and Atkinson (1939), represent the total bile acids, and as the bile acids of the dog can be considered as consisting of about two-thirds cholic acids, the difference of about 1.5 mgm. per 100 ml. found by Josephson and Kaunitz (1937) for these acids in dogs checks rather well.

Reëxcretion of the bile acids. Besides the studies of Tschernoff and Stadelmann, mentioned above, the entero-hepatic circulation of the bile acids has been studied by Greene, Aldrich and Rowntree (1928) and by Whipple and Smith (1928 and 1930). The latter authors found that bile fistula dogs, when deprived of their bile, produced about 100 mgm. bile salts per kilogram body weight per day. The bile salt excretion of these dogs rose to about 800 mgm. per kilogram when the dogs got all their bile back by mouth. The bile salts were then resorbed and again excreted with the fistula bile. They calculated that a dog of about 10 kgm. keeps 7 to 8 grams of bile salts in circulation by resorption and reëxcretion, and that the circulation period of this amount is about 8 to 16 hours. These results have recently been confirmed by Schmidt, Beazell, Berman, Ivy and Atkinson (1939). The circulating amount could be augmented to a certain degree by giving back to the animals more bile salts than they put out. In this way Whipple and Smith (1928) reached a bile salt secretion of about 15 to 17 grams daily, but not more. If still more bile salts were given the surplus was lost. Ivy and co-workers also found a somewhat fluctuating loss of about 10 per cent of bile salts given by mouth in physiological amounts to fistula dogs. They calculated the recovery as the surplus on top of the amount found in the fistula bile, when no bile was given by mouth. This latter

amount was supposed to represent the endogenous bile salt production. However, these authors do not reckon with the possibility that the endogenous bile salt production may be lower when bile acids are amply supplied than when the animal is continuously deprived of all newly made and all secreted bile. For this reason it is possible that the real loss is less than what they found it to be.

In this connection it may be mentioned that Doubilet (1937) found that administration by mouth of cholic acid to bile fistula dogs may depress the deoxycholic output and conversaly deoxycholic acid may decrease the cholic acid excretion by the liver, thus giving the impression of a loss of cholates.

Loss of bile acids and endogenous production. We do not know much about the fate of the small amounts of bile salts lost during the normal circulation. Numerous investigators (see Sobotka, 1937) claim to have found bile acids even in normal urine, but they have all used very unspecific methods. It is probable however that these salts may be excreted by the kidneys to a small extent, at least in jaundice (vide infra) or after injection of large amounts (Lichtman, 1936). Some may be destroyed by the liver (Rosenthal, Wislicki and Pommernelle, 1927; Bollman and Mann, 1933), and some may be decomposed by the intestinal bacterial flora (Licht, 1924). According to the very thorough investigations by Berman, Snapp, Ivy, Atkinson and Hough (1940) there is a possibility that some cholic acids may be oxidised by the organism to dehydro-bile acids (keto acids). In bile fistula dogs these authors found a slight increase of the keto acids in the bile if cholic acids were supplied by mouth. The main loss, however, seems to be the bile salt content of the feces shown in 1862 by Hoppe and in 1863 by Hoppe-Seyler by the means of preparation of cholic acids from dog and cow feces. We fully agree with Sobotka, when he says (p. 37): "Since catabolic destruction of bile acids by the animal organism is exceedingly doubtful, and since their elimination from the body is almost entirely confined to the intestinal route, equilibrium is maintained by synthesis of bile acids paralleling the rate of the fecal losses."

Whipple and Smith (1928) found the endogenous bile salt production of fistula dogs to be about 100 mgm. per 24 hours and kilogram bodyweight. Earlier Foster, Hooper and Whipple (1919) pointed out that bile acid production is highly dependent on the nature and quantity of the food, carbohydrates giving a low production and meat an increase. Especially tryptophan and proline were effective in bile salt production. Cholesterol, which according to its formula might be suspected as the

origin of the bile salts, was completely negative (Foster, Hooper, Whipple, 1919c; Enderlen, Thannhauser, Jenke, 1928). The chemical (structural) relationship between the bile acids and the sex hormones being well known, the biological relationship has been studied by Cuatrecasas and Bruno (1939). In hunger the bile acid production is low but still maintained (Baltaceanu and Vasiliu, 1937). Summing up the investigations in this field, we know, in fact, less than nothing about the origin of the cholic acid part of the bile acids and about the way in which the complicated molecule is being built up.

The endogenous bile salt production in man has been studied in surgical cases with bile fistulas. Greene, Walters and Fredrickson (1930/31) found the daily cholic acid output in such subjects to be 1.0 to 2.3 grams. Collecting the bile by duodenal tubage from 6 cholecystectomized but in other respects normal human subjects, Josephson and Larsson (not published) found the endogenous 24-hour production of cholic acids to be 0.7 to 2.0 grams (average 1.4). Using the same technique but with continuous refeeding of the recovered bile (vide infra) these authors found the daily excretion of 7 subjects to be 1.5 to 7.0 grams. The average was 4.0 or 2.8 times the endogenous production. This means that in man the bile acids take part in the circulation about three times.

Injected bile salts entering the circulation. If bile acids are supplied either by mouth or intravenously to healthy men or animals they very soon enter the normal enterohepatic circulation. At the same time they disappear from the blood almost immediately. Snell, Greene and Rowntree (1927), Bollman and Mann (1936), Chabrol, Cottet and Sallet (1936), Lichtman (1936a), Schmidt (1937) and others, all found a cholate concentration that had returned to normal within less than one hour after a large intravenous bile salt injection. Josephson, Jungner and Rydin (1938) found that most of the injected amount had disappeared already four minutes after the injection. This means that it is too late to catch the injected surplus four minutes after the injection. Normal cats and rabbits after an intravenous injection of, e.g., 250 mgm. cholic acid showed a four minute increase in the blood of only about 6 to 20 mgm. per 100 ml. If the injected amount had been diluted only by the blood of the animal the increase would have been about 100 mgm. per 100 ml. When only 10 mgm. per kilogram body-weight were injected the corresponding increase was 1 to 3 mgm. instead of the calculated 10. Josephson and Larsson (1939) and Josephson (1939a) got similar results on man (fig. 4). Five minutes after the injection of 1000 mgm. cholic

acid the blood content had risen from the normal 2 to 3 mgm. per 100 ml. to 5 to 6 mgm. per 100 ml. and after 500 mgm. it rose to about 4 mgm. per 100 ml. The corresponding dilution values were very approximately about 22 and 12 mgm. per 100 ml. As a rule the blood concentration was normal again after 30 minutes.

In normal individuals the injected salts are soon excreted by the liver. But their first rapid disappearance from the blood cannot be referred solely to this organ. Even if the liver was excluded by ligation of the liver artery and the portal vein, the concentration four minutes after an injection was still only about two-thirds of what might have been expected if the bile salts had been only diluted by the blood (Josephson, Jungner and Rydin, 1938). The most likely explanation of this disappearance is diffusion of the salts into the tissues and fixation there. Bayer (1908) and Chabrol, Cottet and Sallet (1936b) found a fixation of this kind in liver and muscle tissue. On the other hand, Jungner, Rydin and Josephson (1938), in experiments on cats with both obstructive jaundice and liver exclusion, found no disappearance of injected cholates at all. The enormous blood concentrations in these cases, 100 to 400 mgm. per 100 ml. after injection of 250 mgm., roughly corresponded to the dilution of the injected solution by the blood of the animal. Comparison of the results on liver-excluded animals with and without jaundice seems to indicate a fixation mechanism in the normal individual which is not present in jaundice. Possibly this mechanism may be a fixation of the bile salts, which are strongly surface active, to the walls of the blood vessels. This fixation may be of the same type as that found by Verzár and Kuthy (1930), mentioned above, on the walls of the intestine or by Labes and Schlenkert (1932) on red cells and other tissues.

The rapid disappearance of injected bile salts from the blood is normally followed by an almost quantitative excretion by the liver. This has been proved by numerous investigators (see Sobotka and more recently Bollman and Mann, 1936, and Josephson, Jungner and Rydin, 1938). In patients or animals with bile fistulas, 90 to 100 per cent of a not too large amount of injected bile salts is recovered in the bile a few hours after the injection (fig. 1).

The results obtained with bile fistulas may not be considered as true physiological observations. However, Josephson and Larsson (1939) showed that the same rule holds good also when the bile is emptied into the intestine in the normal way. They used six human subjects, who were normal in all respects, except that they had been cholecystectomised one or several years before the experiment. It was necessary to choose

such subjects in order to exclude the function of the gall bladder and to get a continuous bile flow. The bile was quantitatively recovered by means of a double ventriculo-duodenal tube. One gram of sodium

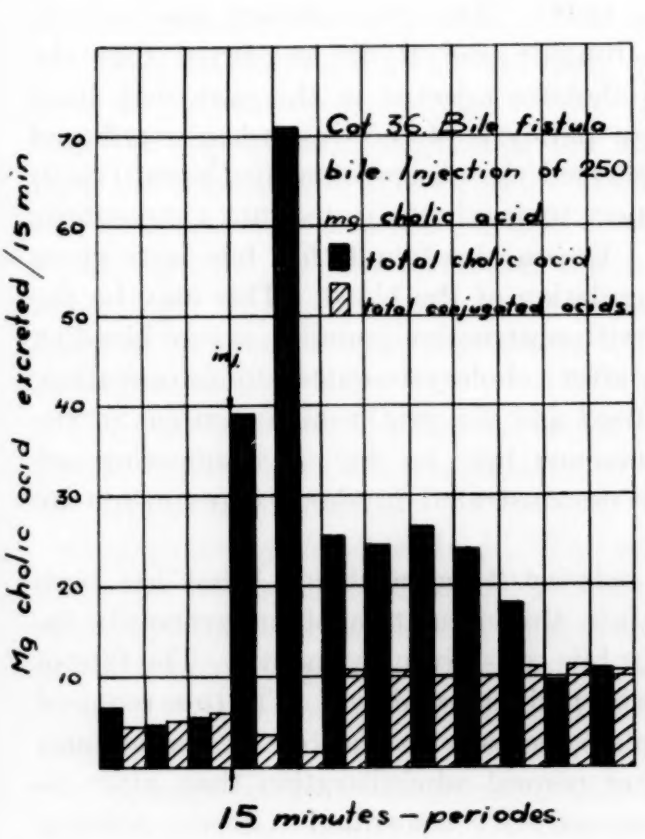

Fig. 1

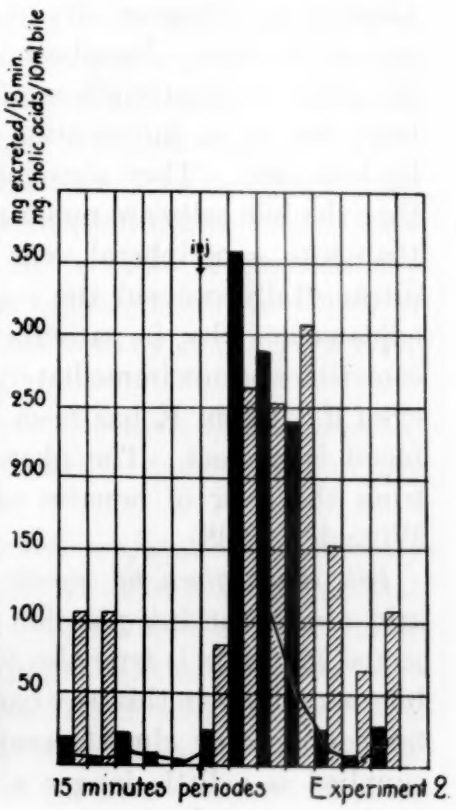

Fig. 2

Fig. 1. From figures of Josephson, Jungner, Rydin (1938). Recovery of cholic acids (black columns) and conjugated bile acids (striped columns) in the bile after intravenous injection of 250 mgm. cholic acid. Two hundred and ten milligram cholic acids recovered in 3 hours. Increase of cholic acid excretion starts immediately after the injection; increase of conjugated acids starts half an hour later.

Fig. 2. From Josephson and Larsson (1939). Recovery by duodenal tubage of cholic acids (black columns) and conjugated bile acids (striped columns) in the bile of a 59 years old, cholecystectomized woman after intravenous injection of 1 gram of cholic acid. The black line is concentration in the bile; 0.88 gram cholic acid recovered in 4 hours. Increase of both cholic and conjugated acids 15 minutes after the injection, but conjugation is incomplete during at least half an hour.

cholate was injected intravenously and after four hours 0.9 to 1.1 gram was recovered in the bile in four cases and 0.5 in two cases (fig. 2).

It seems obvious that the normal liver works very promptly on bile

salts injected intravenously. There is, however, one exception. If the salts are injected in rabbits into a vein of the portal system and thus carried in large concentration directly to the liver, the recovery is very small and the blood concentration remains high for a considerable time (Josephson, Jungner, Rydin, 1938). This phenomenon was not observed in cats. Josephson, Jungner and Rydin suggested that the defective choleretic effect of cholates injected in this way may have been due to an intoxication or paralysis of the liver when overflowed by bile salts. They also confirmed the observation by Doyon (1910) that the bile salts are much more toxic when injected into a mesenteric than into a peripheral vein. Doyon also found that bile salts given intraportally prevent the coagulation of the blood. This may be the explanation why, in patients with obstructive jaundice, severe bleeding sometimes occurs immediately after a cholecysto-enterostomia operation, even if vitamin K has been used and the prothrombin content of the blood is normal. The phenomenon may be due to a squeezing out from the liver of heparin as demonstrated in shock experiments by Wilander (1938).

Bile acids given by mouth entering the circulation. What has been said about the introduction into the circulation of intravenously injected bile salts is true also for bile salts given by mouth. The fate of bile acids given in this way has been discussed above. The time required for complete or almost complete recovery in the bile of the amount supplied is a little longer after peroral administration than after injection owing to the time necessary for resorption (Greene, Aldrich, Rowntree, 1928). Berman, Snapp, Ivy, Atkinson and Hough (1940) recovered about 90 per cent in 8 hours in the bile of natural bile acids given by mouth to bile fistula dogs. The recovery of oxidised (keto-) acids given by mouth was much less and slower.

Bile acids and bile volume. When the bile salt excretion is increased after bile salt supply the bile volume also increases, so that there seems to be a tendency to keep the bile salt concentration rather constant. This choleretic effect of the bile salts is probably the most important regulator of bile volume and bile output under physiological conditions. Doubilet (1937) found that this volume-regulating effect was different for different bile acids even if their effect on bile salt excretion was similar. Thus, using bile fistula dogs, he obtained the most concentrated bile after administration of salts from dog bile. Further the concentration decreased in the order: ox bile, glyco-cholic, cholic, deoxycholic

and dehydrocholic acid.[2] Nevertheless the amount of bile salt excreted was practically the same if equal amounts of the different acids were given.

Schmidt, Beazell, Berman, Ivy and Atkinson (1939) found that the peak output of all bile constituents following a meal with simultaneous supply of bile (imitating the emptying of the gall bladder) does not occur at the same time for bile volume and for bile acids. In fistula dogs the highest concentration of the latter followed markedly later than the maximum of bile output. The conclusion to be drawn from this observation is that the bile acid concentration during the physiological increase in bile formation following a meal is continuously increasing. In this way the concentrated gall-bladder bile which in digestion is first brought into action is followed by a comparatively concentrated liver bile.

The choleretic effect of the bile acids has also been used therapeutically, especially in cases of cholangitis, where a flushing out of the bile capillaries is desired.

Conjugation of the Bile Acids. The normal conjugation. Normally the major part of the circulating bile acids seems to be conjugated, that is, the molecule of cholic acid or related compounds such as deoxycholic acid is coupled to glycine or taurine by a peptid linkage. It has been shown by Schoenheimer, Andrews, Hrdina (1932) and by Colp and Doubilet (1936) that in patients with liver diseases larger amounts of free, unconjugated bile acids may appear in the bile. The question as to whether unconjugated bile acids occur normally is still open. Colp and Doubilet (1936) claimed that in normal human bile only about 80 per cent of the bile acids was conjugated. Josephson and Jungner (1936) found a considerable amount of free acids in bladder bile from several species. It was especially high in rabbit bile and in one pool of guinea-pig bile. In human bladder bile from autopsies they failed to find any free acids.

Conjugation of supplied bile acids. The situation is different if free acids are supplied in large amounts and have to be conjugated in the liver before excretion. Earlier investigators (Foster, Hooper and Whipple, 1919b; Whipple, 1922) emphasized that if much free cholic acid is given to a bile fistula animal it is conjugated in the degree in which there is glycine or taurine available. Not until the supply of these amino acids was exhausted did free cholate appear in the bile. In a recent

[2] This order is also the order of toxicity of the bile acids (Gilbert, 1926).

investigation Josephson, Jungner and Rydin (1938), using comparatively larger amounts of cholic acid, obtained different results. They injected 250 mgm. cholic acid in the form of sodium cholate intravenously into cats and rabbits provided with a cannula in the common bile duct and with the gall bladder ligated. During the first 30 minutes after the injection a comparatively enormous bile salt excretion took place, but nearly all the excreted cholate was unconjugated (fig. 1). Later on, when the bile salt excretion was slowly decreasing, more and more of the cholate appeared in the conjugated form. Corresponding results were obtained by Josephson and Larsson (1939) on human subjects (fig. 2).

The nature of the conjugation. These results might be expected, as the conjugation of the bile salts must be an enzymatic process and thus requires time. Josephson, Jungner and Rydin (1938) even considered this observation to be evidence of the enzymatic character of the conjugation of the bile acids. In fact, Mazza and Stolfi (1932) had earlier claimed that they had prepared an enzyme with this property from liver tissue.

There is, however, also another possible explanation of these results. The time that elapses after an injection of free cholate until the appearance of conjugated acids may be the time required for mobilizing or producing glycine or taurine for the conjugation. The coupling of the free cholic acids is very similar to the formation of hippuric acid from benzoic acid and glycine, and it is not improbable that the same enzymatic apparatus performs both these syntheses, at least in man, where the formation of hippuric acid is considered to take place in the liver. The diminished excretion of hippuric acid after administration of benzoic acid in the Quick test, which is observed in cases with parenchymatous liver diseases, may be referred to an impediment of the enzymatic conjugation, but it may also be referred to a lack of available glycine for the synthesis. Normally the glycine supply seems to be the main regulator of the synthesis (Griffith and Lewis, 1922). That a lack of glycine can be the main obstacle to the hippuric acid formation also when the liver parenchyma is severely damaged was shown by Probstein and Londe (1940), who found that the excretion of this acid increased considerably if glycine was given together with the benzoic acid even to patients with hepatitis. The experience of this laboratory is that lack of glycine is the only cause for a poor hippuric acid production in cases with liver injuries. The Quick test always turned out normally when glycine was given together with the benzoic acid.

The experiments by Foster, Hooper and Whipple (1919b) on bile fistula dogs indicate that a lack of amino acids might in the same way be the reason for non-excretion also of conjugated bile acids. These authors did not determine the total bile acids as they used only the Van Slyke amino nitrogen method (after hydrolysis) for bile acid determinations. They found that cholic acid given by mouth was recovered in greater quantity (as conjugated acid) in the bile if taurine was given together with the bile acid. The same authors also observed that bile fistula dogs produced more conjugated bile acids when given a diet rich in meat than a carbohydrate diet. The experiments of Josephson, Jungner and Rydin (1938) do not answer the question as to whether the excretion of unconjugated bile acids is due to a defective enzymatic conjugation or to the absence of amino acids. New experiments regarding this question are now being carried out in this laboratory.

Bile acid conjugation and protein formation. The rôle of the glycine and the taurine in these conjugation processes, the formation of hippuric acid and that of conjugated bile acids, is very interesting. Considering the question as to why just glycine can be conjugated with benzoic acid we have carried out some further experiments in this laboratory on the formation of peptid linkages. First, we were able to confirm the observation made on rabbits by Griffith and Lewis (1923) and on man by Probstein and Londe (1940), that the excretion of hippuric acid is much higher when glycine is given together with the benzoic acid, than when benzoic acid is given alone. Secondly, we were also able to confirm in man the observation made on rabbits by Griffith and Lewis (1923) that administration of other amino acids with the benzoic acid does not produce any excretion of their benzoyl compounds. So far the amino acids d-alanine and l-histidine have been tested. Thirdly, we are now giving patients with a poor hippuric acid excretion di-peptides containing glycine as one of the components at the same time as the benzoic acid is given. The benzoic acid is, as usual, given by mouth, but the di-peptides are given intravenously in order to prevent their cleavage by the digestive enzymes. It seems as if glycine were the key substance for the formation of peptide linkages and the presence of its free carboxyl group were necessary for the start of the synthesis of a peptide chain. With the carboxyl group of glycine at one end of the chain, more amino acids can always be added to the other end. When a cholic acid or benzoic acid is linked to the amino group of the glycine as in glycocholic or hippuric acid, the building up of the chain is abruptly cut off, as these substances have no amino group. On the

other hand, the free amino-group of glycine does not seem to be sufficient for the start of the synthesis if the carboxyl group is conjugated. The experiments are being continued and will be published later.

The Continuity of the Bile Acid Excretion. Diurnal variations in the liver function. Very little is known about the continuity of the excretion and formation of the bile acids. Forsgren (1928) discovered the rhythm of the liver function. His discovery was followed by a series of investigations in this field, mostly in Swedish laboratories. Using histological methods Forsgren (1928) found that the glycogen content of the liver of rabbits was highest during the night and in the early morning, the assimilatory phase. The content of what he called bile constituents was highest during the day and evening, the secretory phase. There were in different animals slight variations in the hours when the phases appeared (Forsgren, 1930). The bile constituents were demonstrated only by the formation of microscopically visible precipitation with barium chloride, and for this reason it cannot be considered as proved that they included the bile acids. However, the results have been confirmed later by different methods. The rhythm of the glycogen content has been demonstrated by Ågren, Wilander and Jorpes (1931) and by Sjoegren, Nordenskjoeld, Holmgren and Moellerstroem (1938). The latter investigators found the variations to be largely dependent on food intake. Using chemical methods for the study of the variations of the blood sugar concentration in man, Moellerstroem (1930) concluded that a similar rhythm occurs also in the human liver. Sechel and Kato (1938) using newborn rats and the Forsgren methods found no liver rhythm during the first three weeks of life. After that age the rhythm was developed. Holmgren (1936) in an extensive histological study of the rhythmic changes in the resorption from the intestines, found variations of the fat content in the intestinal mucosa speaking in favour of Forsgren's opinion that there are rhythmic changes also in the bilt salt secretion. These changes are probably followed by changes in the resorption of fat acids, and in this way they have an influence on the histological picture of the mucosa. Higgins, Berkson and Flock (1933) emphasized that the cyclic changes in the rat liver are entirely due to the time of food intake. They were able to change the time of the maximum and minimum of the liver glycogen by changing the time of the meals. But even from their experiments some endogenous cyclic changes can be inferred.

There are some direct observations published on the daily variations in bile flow and bile acid excretion in patients and animals with bile

fistulas. In 1889 Copeman and Winston and in 1897 Pfaff and Balch found that patients with bile fistulas produced more bile during the daytime and evening than during the night and morning. These observations are in conformity with the later observations by Forsgren (1928). They did not include bile acid analysis. Foster, Hooper and Whipple (1919a) mentioned that the bile salt output of bile fistula dogs is somewhat higher in the morning than in the evening.

Josephson and Larsson (1934) studied a bile fistula patient. Concerning the variations in the bile volume they confirmed the earlier result, but they found another rhythm in the bile acid output. In this patient a weak excretion maximum was observed about midnight. The variations in bile volume output in man have later been confirmed by Koster, Shapiro and Lerner (1936) and by Zuckermann, Kogut and Jacobi (1939).

Diurnal variations of the bile acid secretion. All studies of the variations in the bile salt excretion of bile fistula subjects have the drawback that they do not correspond to physiological conditions when the bile acids are circulating. In the hope of approaching what normally occurs, Josephson and Larsson (not published) undertook some experiments in this field. They used human subjects, on whom a cholecystectomy had been carried out some years earlier. They chose cases in which the operation had been without complications, and which were quite normal when the experiments were carried out. The use of cholecystectomized patients was necessary in order to obtain the liver bile constantly without interruptions from the gall bladder. The bile was continuously collected by weak suction through a double duodenal tube with one of the branches in the stomach and one in the duodenum. The subjects fasted throughout the experiments, which lasted during 24 to 30 hours.

In the first series of experiments the bile was continuously withdrawn. In another series on the same subjects the bile was collected during 30 minutes each hour, a sample was taken for analysis and the bile was slowly reinjected through the duodenal tube during the other 30 minutes of the same hour. In this way a physiological entero-hepatic circulation could be maintained during the whole experiment. At the same time the bile was continuously analysed and quantitatively measured.

In both series the values varied extremely from hour to hour and no typical or constantly returning maxima or minima could be found. However, the excretion of bile and cholates tended to be greater during

the day, especially the forenoon, than during the night. This difference was observed in both series but was more striking in the experiments where the bile was reinjected. Consequently, there is apparently a rhythmic variation, at least of the excretion or entero-hepatic circulation of the cholates in man, principally of the type emphasized by Forsgren (1928). If this rhythm is to be credited variations in the liver function or in the resorption from the intestine cannot be definitely decided, but the latter reason seems to be the most probable one, as the bile acids are very soon reëxcreted when once introduced into the blood, as described above. The existence of rhythmic variations in the endogenous bile acid production is very doubtful.

The results arrived at by Josephson and Larsson will be published later in full detail, but a picture of their results concerning the excretion of the bile acids is demonstrated in figure 3, showing two typical experiments.

Liver Diseases and Bile Acid Circulation. Bile salt production in jaundice. In jaundice, whether obstructive or caused by parenchymatous damage of the liver cells (hepatitis), the entero-heptic circulation of the bile acids is more or less completely broken.

In obstruction the liver cells still maintain their ability to produce and to transport bile acids. Their concentration in the blood is increased and they also appear in the urine. If the liver parenchyma is injured the bile acid production decreases. Thus the bile acid excretion in the urine of dogs with the bile ducts ligated is diminished if the liver cells of the animals are damaged by poisons, for instance, by carbontetrachloride (Bollman and Mann, 1935) and the bile salt excretion by bile fistula dogs is also diminished by such poisons (Whipple and Smith, 1930). A similar reaction is observed in patients with disorders of the bile ducts or the gall bladder. After operation including the establishment of a bile fistula these patients usually have a very low bile salt production during the first three or four days following the operation (Colp and Doubilet, 1936; Kohlstaedt and Helmer, 1937; Grey, Butsch and McGowan, 1938). When the liver has recovered the production is normal again.

In jaundice there is usually an increased concentration of the bile acids in the blood. The figures published by the numerous investigators on this subject (Snell, Green, Rowntree, 1927; Katayama, 1928; Fuentes, Apolo and Esculies, 1930; Bollman and Mann, 1935; Jungner, Rydin and Josephson, 1938, and others) vary considerably, according to the different methods of analysis. Usually the figures are higher in ob-

structive jaundice (up to 30 mgm. per 100 ml.) than in hepatitis (up to 10 mgm. per 100 ml.) partly owing to the maintained ability of the

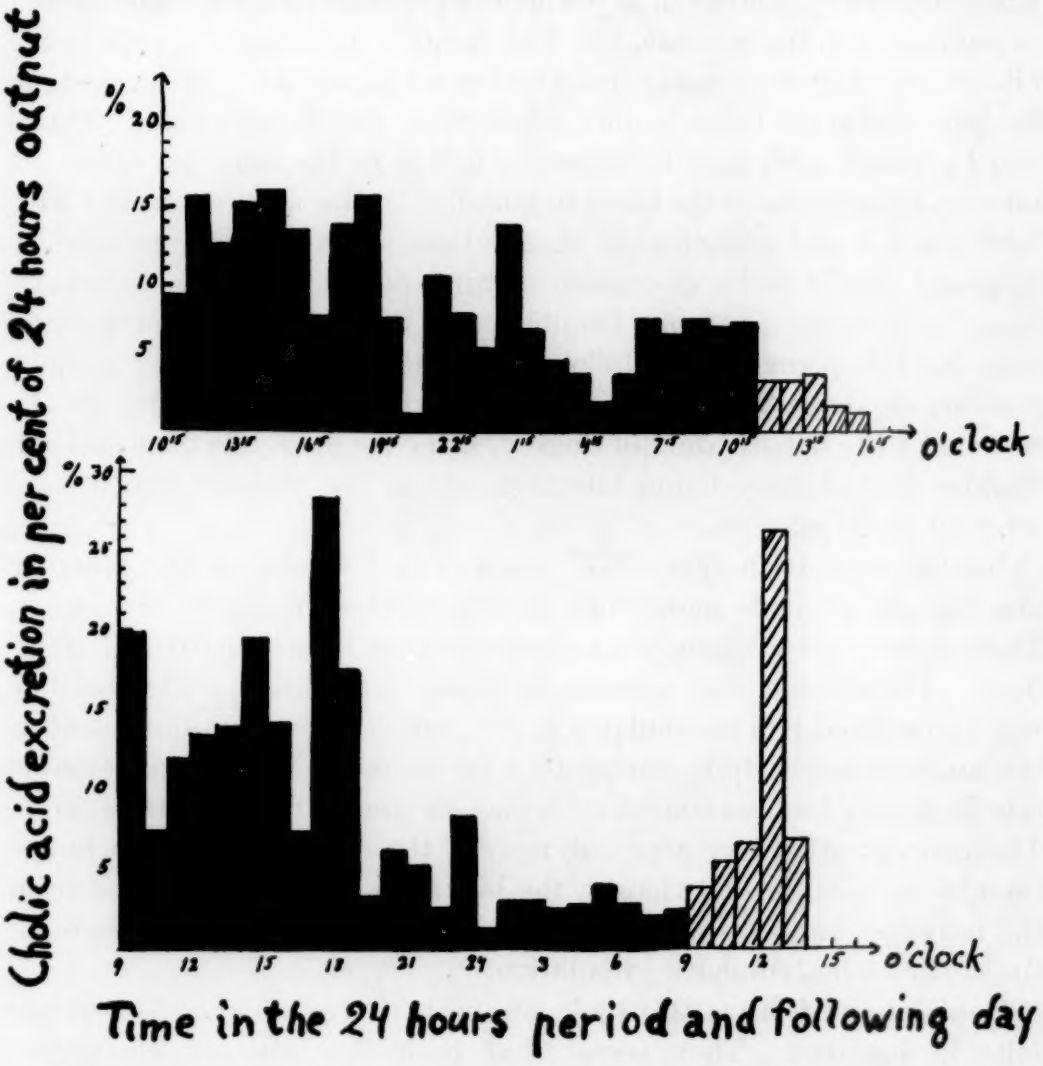

Fig. 3. From Josephson and Larsson (not published). Diurnal excretion of cholic acids in two healthy cholecystectomized women, one 26 years old (above) and one 18 years old (below). The bile was collected by duodenal tube during the first 30 minutes of each hour. During the other 30 minutes, after a sample had first been taken, the bile was slowly re-injected into the duodenum, thus maintaining the normal circulation. Both experiments lasted for 29 hours, the first 24 demonstrated by black columns, the last 5 by striped columns. During this period the patients were in bed and got neither food nor drink. Considerably more cholic acids excreted during the day than during the night.

liver cells in obstruction to produce bile salts and partly to the hepato-bilio-lymphatic circulation appearing only in obstruction which carries the bile salts from the liver to the circulating blood.

Bile salt circulation in jaundice. In cases of obstruction a new circulation route takes the place of the entero-hepatic one. Mendel and Underhill (1905), and even investigators previous to them, proved that in animals with the common bile duct ligated, substances present in the bile (injected dyes or milk) could be traced along the lymph vessels of the liver and in the thoracic duct, where these vessels open out. Mendel and Underhill consequently suspected this to be the route by which the bile constituents reach the blood in jaundice. This is in conformity with later clinical and pathological observations described for instance by Eppinger (1937) in his discussion of what he calls "icterus e dissociatione." Recently Shafiroff, Doubilet and Ruggiero (1939) have found that the bile pigments can follow the same route. Applying a slight pressure of about 30 cm. water (in the form of a bile column) on the content of the hepatic duct of dogs with the common bile duct and gall bladder ligated, they found bile pigments in the thoracic duct lymph after 10 to 20 minutes.

Josephson and Kaunitz (1937) showed that in obstructive jaundice also the cholic acids make such a hepato-bilio-lymphatic circulation. These authors used dogs in acute experiments with a fistula to the thoracic duct. They found that animals in which the normal outflow of bile was maintained had no cholates in the lymph. On the other hand, if the animals were icteric, owing to a previous ligation of the common bile duct, they found a considerable cholate concentration in the lymph. This concentration increased still more if these dogs were given taurocholates by mouth. Obviously, the bile acids had been resorbed from the intestine, carried to the liver by the portal blood and had entered the hepato-bilio-lymphatic circulation.

Doubilet and Colp (1936) spoke of another form of circulation of bile salts in jaundice. They found that in cholecystitis or cholangitis about nine-tenths of the bile salts, especially the cholates, was reabsorbed from the gall bladder and the bile ducts and again carried to the liver. Normally, this simple bilio-hepatic circulation does not occur. In cholecystitis it takes up to 90 per cent of the cholates.

In hepatitis and other forms of damage to the liver cells themselves the diseased cells form a barrier between the blood and the bile capillaries. For this reason no circulation of bile constituents whatsoever takes place. This may possibly be one of the reasons why the bile acids in the bile from fistula dogs decrease when the animals are given carbontetrachloride or other liver-poisons. Of course a more important reason may be diminished bile salt formation in hepatitis.

The difference between the circulation of the bile salts in obstruction and the blockage in hepatitis may be illustrated by the different behavior of cholates injected intravenously.

If bile salts are injected intravenously into icteric animals or patients the resulting increase of the cholates in the blood is much higher and lasts longer than is normally the case (Snell, Green and Rowntree, 1927; Chabrol, Cottet and Sallet, 1936a; Bollman and Mann, 1936; Jungner, Rydin and Josephson, 1938). However, also in this case the main part of the injected salts very soon disappears from the blood. The fate of the bile salts after their disappearance has been the subject of several theories; Rosenthal, Wislicki and Pommernelle (1927) and Bollman and Mann (1936) claim that the acids are partly destroyed by the liver. Bollman and Mann (1933) regard their observation that supplied bile acids appeared in the urine after complete removal of the liver but not when the liver was intact and the bile duct ligated, as in favour of their theory of destruction in the liver. However, their urine method is extremely unspecific. Chabrol, Cottet and Sallet (1936b) recovered most of the injected salts in the liver tissue of the jaundiced animal and concluded that the liver has a special property of fixing and storing the bile salts. Finally, as indicated by the Hay test, the surplus may be excreted by the kidneys. Thus, attempts to determine the bile salts in the urine have been made by numerous authors with positive results (Bollman and Mann, 1936; Lichtman, 1936), but they all used too unspecific methods of analysis. According to Lichtman the cholates should pass the kidneys easier than the deoxycholates, thus causing a concentration of deoxycholic acid in the blood higher than that of cholic acid.

Jungner, Rydin and Josephson (1938) claimed that injected bile salts in obstructive jaundice are absorbed by the liver according to Chabrol, Cottet and Sallet (1936b) but that little by little they subsequently enter the hepato-bilio-lymphatic circulation. This is the reason why injected bile salts disappear comparatively quickly from the blood in obstruction. In hepatic jaundice on the other hand where there is no bile circulation they remain for a long time in the blood. An adsorption to the walls of the blood vessels (as mentioned above) does not seem to take place in jaundice, at least not in the same degree as normally. This is supported by the above-mentioned observations of Josephson, Jungner and Rydin that if an injection of bile salts is undertaken on an animal with the liver excluded and having a considerable icterus due to a previous ligation of the bile duct, the blood con-

centration will rise enormously and roughly correspond to the simple dilution of the injected solution by the blood of the animal. In these cases the walls were already loaded with bile salts owing to the ligation and the subsequent jaundice.

Diagnostic use of bile acid elimination. From a diagnostic point of view Jungner, Rydin and Josephson (1936) studied the elimination from the blood of cholic acids injected intravenously into animals with several forms of experimental jaundice (ligation, carbontetrachlorid, phosphorus). They found that in obstruction the injected bile acids disappeared from the blood rather quickly. Sometimes a new, slight increase occurred again about one hour after the injection. Jungner, Rydin and Josephson (1936) explained this by the maintenance of the hepato-biliolymphatic circulation, by which the bile salts are again carried to the blood, thus causing the second peak. In hepatitis the decrease of the cholate concentration of the blood was rather slow after the first rise in connection with an injection.

Josephson (1939a) has tried to utilise these different types of reaction in a new liver function test on patients with icterus. He injected 10 ml. of a 0.52 per cent sodium cholate solution (corresponding to 0.5 gram cholic acid) intravenously into patients with disorders of the liver or bile ducts. The solution also contained 25 per cent glucose, which prevents the burning sensation following an injection of a pure cholate solution. The blood cholates were determined both before and 5, 30 and 60 minutes after the injection. It turned out that the disappearance of the cholate from the blood in the different types of liver disease was in conformity with the results observed in animals with experimental liver injuries. Some typical results are shown in figure 4.

In cases with liver disease without jaundice (some cases of liver cancer, cholecystitis and cirrhosis) the cholates disappeared quite as rapidly as in normal subjects. After 5 minutes only a slight elevation could be found and after 30 minutes the blood concentration was normal again. In bile obstruction the increase 5 minutes after the injection was usually obvious, 5 to 8 mgm. per 100 ml., but after 30 minutes the concentration had already fallen to the original level or almost so. In cases with damage of the liver parenchyma (mainly hepatitis) on the other hand the first increase after the injection was usually of the same order of magnitude as in obstructive jaundice. The subsequent decrease of cholates, however, was very slow in hepatitis in contrast to its behaviour in obstruction. Even one hour after the injection the concentration of the blood cholates in hepatitis was still nearly as high as after 5

minutes. From this it may be assumed that the rate of the elimination of injected cholate may be used in the differential diagnosis between obstructive jaundice and disease of the liver parenchyma such as hepatitis. A difference between the concentration 5 minutes after

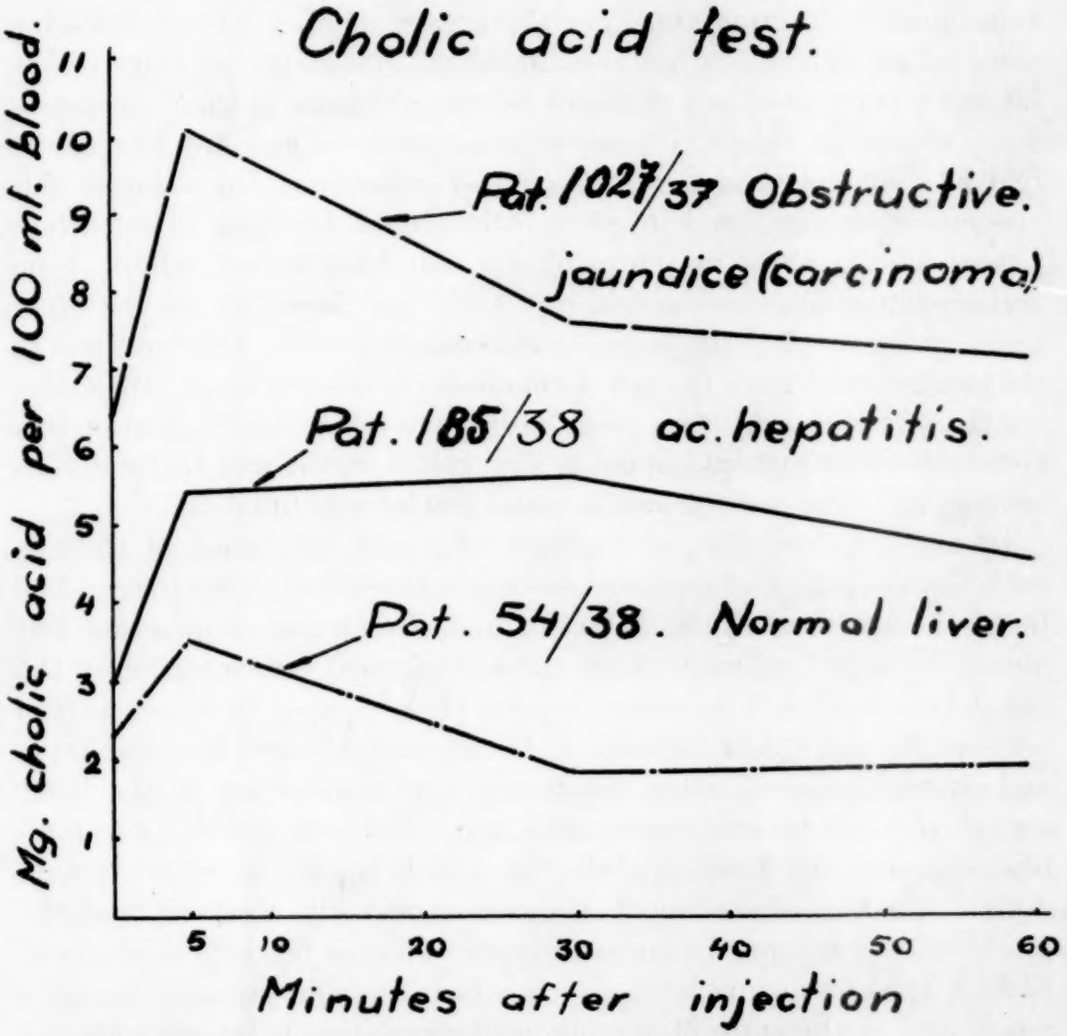

Fig. 4. From figures by Josephson (1939a). Cholic acid tolerance test. Blood concentration of cholic acids before and 5, 30 and 60 minutes after the intravenous injection of 0.50 gram cholic acid in human subjects. The curves represent three typical cases.

the injection and that after 30 minutes of more than 2 mgm. per 100 ml. indicates an obstruction, while a difference of less than 1 mgm. per 100 ml. indicates a parenchymatous disease. These differences are limit values. Usually the difference between the two types of elimination is much more obvious.

Since the publication of this bile salt tolerance test the results have been confirmed in this laboratory by further observations on other cases. Unfortunately, however, the bile salt determination method as described by Josephson (1935) will scarcely have any future in clinical work as the cholate determinations are far too complicated and time-consuming for routine use. For this reason another way of following the cholate elimination has been achieved, the surface-activity of the bile acids being used as a means of following changes in their concentration. The method is very nonspecific and does not give any idea of the real bile salt concentration of the blood. However, the value of this absolute concentration is of very little clinical interest. The surface tension of the blood or serum of one individual is very stable if no surface-active substance is added. A bile salt injection, on the other hand, causes an obvious change in the surface-tension, which returns to the original level when the salt is eliminated from the blood. By studying the rate of this return it seems possible to follow the elimination in a convenient way without obtaining any values of the real bile salt concentration. The method will be published later in full detail.

In cases of liver diseases the formation and circulation of the bile acids seem to be rather independent of other functions of the liver. The blood-cholates are seldom increased and the cholate elimination test always turns out normally when the bile pigment concentration of the blood is normal, but in other respects there is very little parallelism between the amount of bile acids in the blood or bile and the production and concentration of other substances with connection to the liver, e.g., cholesterol, plasma protein, urea, etc. Even the plasma prothrombin seems to be independent of the bile acids in the intestines to a certain degree. In this laboratory it has been repeatedly observed that the prothrombin level may be normal in spite of a poor bile acid production. In fact, the most convenient way in which the clinician may obtain a rough idea of the state of the bile acid circulation is by studying the feces as the fat resorption is very susceptible to disorders of the bile acids in the intestines. The drawing of conclusions concerning the production or circulation of the bile acids from results of investigations of other liver functions may easily give erroneous results.

REFERENCES

ABE, Y. J. Biochem. **26:** 323, 1937.
ÅGREN, G., O. WILANDER AND E. JORPES. Biochem. J. **25:** 775, 1931.
BALTACEANU, G. AND C. VASILIU. Compt. rend. soc. biol. **126:** 715, 1937.
BAYER, G. Biochem. Ztschr. **13:** 215, 1908.

BERMAN, A. L., E. SNAPP, A. C. IVY, A. J. ATKINSON AND V. S. HOUGH. Am. J. Digest. Dis. **7**: 333, 1940.
BOLLMAN, J. AND F. MANN. Arch. Pathol. **16**: 304, 1933.
Ann. Int. Med. **9**: 617, 1935.
Am. J. Physiol. **116**: 244, 1936.
CHABROL, E., J. COTTET AND J. SALLET. Paris médical **26**: 428, 1936a.
Compt. rend. soc. biol. **122**: 186, 1936b.
COLP, R. AND H. DOUBILET. Arch. Surgery **33**: 913, 1936.
COPEMAN, M. AND W. B. WINSTON. J. Physiol. **10**: 213, 1889.
CUATRECASAS, J. AND A. BRUNO. Rev. Sud-Amer. Endocrinol. **22**: 86, 1939.
DOUBILET, H. Proc. Soc. Exper. Biol. and Med. **36**: 687, 1937.
DOUBILET, H. AND R. COLP. Proc. Soc. Exper. Biol. and Med. **34**: 326, 1936.
Arch. Surgery **34**: 149, 1937.
DOYON, M. J. de Physiol. **12**: 197, 1910.
ENDERLEN, E., S. THANNHAUSER AND M. JENKE. Arch. exper. Path. u. Pharmakol. **130**: 308, 1928.
EPPINGER, H. Die Leberkrankheiten. Vienna, 1937.
FORSGREN, E. Skand. Arch. Physiol. **53**: 137, 1928.
Skand. Arch. Physiol. **59**: 217, 1930.
FOSTER, M. G., C. W. HOOPER AND G. H. WHIPPLE. J. Biol. Chem. **38**: 367, 1919a.
J. Biol. Chem. **38**: 379, 1919b.
J. Biol. Chem. **38**: 421, 1919c.
FRÖLICHER, E. Biochem. Ztschr. **283**: 273, 1935/36.
FUENTES, V., E. APOLO AND J. ESCULIES. Ztschr. exper. Med. **73**: 412, 1930.
GILBERT, E. Ztschr. exper. Med. **52**: 778, 1926.
GREENE, C., M. ALDRICH AND L. G. ROWNTREE. J. Biol. Chem. **80**: 753, 1928.
GREENE, C., W. WALTERS AND C. FREDRICKSON. J. Clin. Investigation **9**: 295 1930/31.
GREGORY, R. AND T. A. PASCOE. J. Biol. Chem. **83**: 35, 1929.
GREY, H., W. BUTSCH AND J. MCGOWAN. Arch. Surgery **37**: 609, 1938.
GRIFFITH, W. AND H. LEWIS. J. Biol. Chem. **57**: 1, 1923.
HIGGINS, G., J. BERKSON AND E. FLOCK. Am. J. Physiol. **105**: 177, 1933.
HOLMGREN, H. Studien über 24-stundenrythmische Variationen des Darm-, Lungen- und Leberfettes. Helsingfors, 1936.
HOPPE, F. Virchow's Arch. **25**: 181, 1862.
HOPPE-SEYLER, F. Virchow's Arch. **26**: 519, 1863.
JENKE, M. UND U. GRAFF. Klin. Wchnschr. **18**: 125, 1939.
JOSEPHSON, B. Biochem. J. **29**: 1519, 1935.
J. Clin. Investigation **18**: 343, 1939a.
Klin. Wchnschr. **18**: 1280, 1939b.
JOSEPHSON, B. AND G. JUNGNER. Biochem. J. **30**: 1953, 1936.
JOSEPHSON, B., G. JUNGNER AND A. RYDIN. Acta Med. Scand. **97**: 237, 1938.
JOSEPHSON, B. AND H. KAUNITZ. Ztschr. exper. Med. **102**: 195, 1937.
JOSEPHSON, B. AND H. LARSSON. Skand. Arch. Physiol. **69**: 227, 1934.
Acta Med. Scand. **99**: 140, 1939.
JOSEPHSON, B. AND A. RYDIN. Biochem. J. **30**: 2224, 1936.
JUNGNER, G., A. RYDIN AND B. JOSEPHSON. Acta Med. Scand. **97**: 254, 1938.

KATAYAMA, I. Arch. int. Med. **42**: 916, 1928.
KOHLSTAEDT, K. G. AND O. M. HELMER. Am. J. dig. Dis. and Nutr. **4**: 306, 1937.
KOSTER, H., A. SHAPIRO AND H. LERNER. Am. J. Physiol. **115**: 23, 1936.
v. KUTHY, A. Klin. Wchnschr. **14**: 308, 1935.
LABES, R. AND T. SCHLENKERT. Arch. exper. Path. u. Pharmakol. **166**: 186, 1932.
LICHT, H. Biochem. Ztschr. **153**: 159, 1924.
LICHTMAN, S. Am J. Physiol. **117**: 665, 1936.
Am. J. Physiol. **124**: 94, 1938.
MAZZA, F. P. AND G. STOLFI. Arch. Scienze Biol. **17**: 434, 1932.
MENDEL, L. AND F. UNDERHILL. Am. J. Physiol. **14**: 252, 1905.
MOELLERSTROEM, J. Acta Soc. Medic. Suecanae **56**: 1, 1930.
PETTENKOFER, M. Ann. der Pharmacie **52**: 90, 1844.
PFAFF, F. AND A. BALCH. J. exper. Med. **2**: 49, 1897.
PROBSTEIN, J. G. AND S. LONDE. Ann. Surgery **111**: 230, 1940.
RIEGEL, C., K. O'SHEA ELSOM AND I. S. RAVDIN. Am. J. Physiol. **112**: 669, 1935.
ROSENTHAL, F., L. WISLICKI AND H. POMMERNELLE. Arch. exper. Path. u. Pharmakol. **122**: 159, 1927.
SCHIFF, M. Pflüger's Arch. **3**: 598, 1870.
SCHMIDT, L. H. Am. J. Physiol. **120**: 75, 1937.
SCHMIDT, C. R., J. M. BEAZELL, A. C. BERMAN, A. C. IVY AND A. J. ATKINSON. Am. J. Physiol. **126**: 120, 1939.
SCHMIDT, L. A. AND A. E. DART. J. Biol. Chem. **45**: 415, 1920/21.
SCHOENHEIMER, R., E. ANDREWS AND L. HRDINA. Hoppe-Seyler's Ztschr. **208**: 182, 1932.
SCHWIEGK, H. Arch. exper. Path. u. Pharmakol. **168**: 693, 1932.
SECKEL, H. P. G. AND K. KATO. Arch. Path. **25**: 347, 1938.
SHAFIROFF, B. G. P., H. DOUBILET AND W. RUGGIERO. Proc. Soc. exper. Biol. and Med. **42**: 203, 1939.
SJOEGREN, B., T. NORDENSKJOELD, H. HOLMGREN AND J. MOELLERSTROEM. Pflüger's Arch. **240**: 427, 1938.
SNELL, A., C. GREENE AND L. ROWNTREE. Arch. Int. Med. **40**: 471, 1927.
SOBOTKA, H. Physiological chemistry of the bile. Baltimore, 1937.
STADELMANN, E. Ztschr. Biologie II **16** (34): 1, 1896.
TAPPEINER, H. Sitz.-Ber. Akad. Wissensch., Vienna **77**: 281, 1878.
TSCHERNOFF, W. Virchow's Arch. **98**: 231, 1884.
VERZÁR, F. AND A. v. KUTHY. Biochem. Ztschr. **205**: 369, 1929.
Biochem. Ztschr. **230**: 451, 1930.
WHIPPLE, G. H. Physiol. Reviews **2**: 440, 1922.
WHIPPLE, G. H. AND H. P. SMITH. J. Biol. Chem. **80**: 697, 1928.
Biol. Chem. **89**: 727, 1930.
WIELAND, H. AND H. SORGE. Hoppe-Seyler's Ztschr. **97**: 1, 1916.
WILANDER, O. Skand. Arch. Physiol. **81**: suppl. 15, 1938.
ZUCKERMAN, C., B. KOGUT AND M. JACOBI. Am. J. Dig. Dis. and Nutr. **6**: 183, 1939.

THE PHYSIOLOGY OF THE GENE

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In textbooks of physiology, it is customary to treat the cell as the ultimate living unit. The properties of the cell are interpreted as resulting from the interactions of individually non-living substances when organized in the physical system characteristic of protoplasm. A different treatment is found in genetics. The ultimate unit of life here is not the cell but the gene. The purpose of this paper is to review briefly certain aspects of the rôle of genes in cellular physiology. Its scope forbids any attempt at an exhaustive review of the pertinent literature (cf. 101, 256).

The Gene as a Unit of Heredity. The gene concept is based primarily on results from breeding experiments. It is found that most of the differences among individuals of a species can be interpreted successfully as due to combinations of differences in numerous separable components of a postulated hereditary material; and in the environments. A gene, from this standpoint, is one of a number of alternative conditions of a hereditary component, occurring in an individual only, as a rule, if in continuous unchanging existence along one of the ancestral lines; incapable of resolution by any known experiment into separately transmitted subentities, transmissible asexually to all offspring, but sexually only to a certain proportion (usually 50 per cent). The data directly imply the existence of numerous intracellular entities, each capable of synthesizing exact duplicates irrespective of the nature of the rest of the cell or of the organism or of the external environment, and producible only by such synthesis. The differential effect of any pair of alternative genes (alleles) on the characteristics of individuals is constant among all individuals in which the other conditions (genetic and environmental) are the same, however variable, apparently, in intervening generations in which these other conditions have not been controlled. The most important qualification of these principles is that a gene may occasionally undergo an abrupt change (mutation) to an alternative condition, which thereafter persists and multiplies as of the new sort. The likelihood of undergoing such a change may be increased by certain environ-

mental conditions (treatment with ionizing radiations, high temperature, etc.) but not apparently in a directed fashion. Mutation rate is also sometimes increased by particular associated genes (56, 211). Finally it may be noted that genes differ greatly in stability, the rate of mutation in most cases being apparently less than 10^{-5} per generation but with much higher rates in the relatively rare class known as unstable genes.

The unitary character of genes may be illustrated by an experiment (196) in which more than 300 successive back crosses of long winged flies of the species Drosophila melanogaster to a pure vestigial winged stock have not modified in any way the character long wing or its frequency (50 per cent) in each generation, although in the 300th generation the flies were only $(\frac{1}{2})^{300}$ long winged by ancestry. The autonomy of genes is especially strikingly illustrated in experiments in which genes from one species have been introduced into another by repeated back crossing to the latter (59, 266).

The Gene as a Physical Entity. A great deal has been deduced about the nature of the system of genes without recourse to any other technique than that of breeding, but, while adequate, these deductions take on a greater appearance of reality when it is found that they are exactly paralleled in all respects by the visible behavior of the thousands of individualized granules (chromomeres) distributed along the unbranched, threadlike chromosomes. A single set of the latter is typically present in germ cells, a double set in the somatic cells, but other numbers are found in just those cases in which the breeding results indicate corresponding other numbers of alleles. The number of pairs of chromosomes agrees with the number of linkage systems. Visible deficiencies, duplications, translocations and inversions occur in the chromosomes where the breeding data indicate that such changes have occurred in the linkage systems.

Until recently the breeding technique has given a much more detailed account of the architecture of heredity than has the microscope but since Painter's discovery of the relationship between individual genes and identifiable granules in the giant chromosomes of the salivary glands of Drosophila (192) the stimulatory effects of discoveries in each field on the other have been more nearly equal.

These chromosomes have an aggregate length of 1 to 2 mm., depending on the amount of stretch, or more than 100 times that of the chromosomes seen at metaphase of mitosis. They show some 5000 identifiable bands of chromatin, separated by more extensive, nonstaining material (27, 28). They are believed to be cylindrical bundles containing a

large number of stretched out replications of the two homologous basic chromosomes with corresponding chromomeres associated to form the bands (cf. 193, 170). Particular genes have been demonstrated to be located on or near particular bands by careful observation of the structure near the ends of the loops and bulges found where one homolog has an inversion, duplication or deficiency (covering known genes) relative to the other homolog. The number of bands agrees roughly with estimates of the total number of genes in Drosophila and there may be a one to one relation. In a study of minute deficiencies induced by x-rays, 4 distinguishable genes (yellow, achaete, scute, lethal) were found to be located in what then seemed a broad single (or perhaps double) band (the second from the distal end of the X chromosome (184)). A visible deficiency of half of this band (some 125 mμ) gave viable flies showing the recessive character yellow and achaete. Later observations, however, showed four visible components in this band (81, 27).

There is some ambiguity in the terms used by geneticists. As noted, the breeder, in referring to a gene, means merely a member of a set of alternative *conditions* in the hereditary material. Whether the condition in a particular case is one of addition, of loss of material, or of rearrangement, he does not ordinarily know. In cytogenetics and physiological genetics, however, it becomes desirable to be able to refer to the actual material at a certain locus as it is when affected by a particular allele. The term gene has come to be used also in this sense.

The question whether the genes in this sense are discrete entities or merely regions in a continuum is an old one. Breeding data merely prove that a certain difference behaves as unitary in the tests that have been applied, but there can be no assurance that such a difference may not turn out to be composite by other tests. From this viewpoint, genes are merely regions of the chromosome within which crossing over or other breakage has so far not been observed to occur.

There are a number of possibilities both with respect to the physical and physiological discreteness of the genes (cf. 32, 101).

1. The genes may be physical units, separated from each other by non-genic material within which exchange at crossing over or breakage under x-ray treatment is likely to occur.

2. The genes may be physical units in immediate contact with each other, the breaks occurring only between genes.

3. The genic material of a chromosome may be a continuum, capable of breakage at any point.

If the gene is a physical unit, it may act as a physiological unit, but it is also possible that it may be composite physiologically or that a group of adjacent genes may act together differently than if more widely separated. If the genic material is a continuum, there may be more or less widely spaced centers of physiological activity separated by relatively inert material or there may be overlapping regions that act as physiological units.

The strongest genetic argument for a high degree of physical unity in the genes is probably the fact that crossing over does not produce the mutational changes that would necessarily occur if there were any inequality in the points of exchange (239). Study of breakages near the distal end of the X-chromosome of Drosophila under x-ray treatment indicated that in this case also the points of breakage tended to be repeated exactly (184).

The idea that genes may be separated by nongenic material encounters one difficulty. The essential difference between genic and nongenic material is obviously the capacity of the former and incapacity of the latter to synthesize duplicates. But if the genes are separated by nongenic material the new genes formed when the system duplicates might be expected to fall apart which, of course, is not the case except for crossovers, occurring perhaps about once in a thousand times between adjacent genes. The persistence of an order is known not to be due to specific attraction of adjacent genes, since new orders due to translocation, inversion, duplication or deficiency persist as strongly as the old order, once they have been brought about. Either there must be continuity of the genic material, or nongenic material must immediately collect between new genes, perhaps produced by them, to bind together those which happen to be adjacent. The latter is postulated in Belling's theory of crossing over (18). The connections between genes cannot rest on polarity, since inversions of order of blocks of genes occur (237).

There is considerable correlation between the gene as a block within which crossing over does not occur and the gene as an apparent physiological unit. Multiple alleles in general affect the same characters and frequently seem to differ only in the degree of effect (review 232). On the other hand, there is little or no tendency for genes that are close together in the linkage system to be similar in effect (e.g., yellow and achaete seem to be adjacent genes in Drosophila (184) but one has its principal effect on body color, the other on development of the bristles). In general the effect of replacing one allele by another is as independent of replacement in neighboring loci as in any other parts of the genetic

system. The translocation of a gene, whether type or mutant, to another region usually has no apparent effect on its physiological activity.

These principles are not, however, without exception. It is now clear that the effects of multiple alleles are by no means always merely differences in degree (232) and in some cases the effects appear to be on unrelated characters, e.g., spineless and aristapedia in Drosophila (241).

In some cases, as first shown by Sturtevant (239), the effect of a gene is demonstrably affected by its position in the system (review 61). Thus, chromosome aberrations in Drosophila often result in character changes (especially changes in viability (195), in stability of effect (177, 58, 217, 107) and in dominance (65)) even when there is no change as far as known in the genic material except rearrangement. In many of these cases it is possible to interpret the results as due to occurrence of a mutation or to loss of genic material near the break but enough cases are known in which a restoration of the original arrangement or a return of an apparently affected gene to its normal position has brought about a return to the normal character to make it certain that position effect is real (239, 65, 66, 194, 110, 57). Only one clear case, however, seems to have been demonstrated in plants (41) in spite of numerous translocations. In these position effects there is, in a sense, an overlapping of the genes as physiological units, but this may mean no more than that interactions between immediate gene products are facilitated by proximity (239, 187).

The appearance of the chromosomes when drawn out to maximum lengths (prophase, and resting phase as seen in the salivary glands of diptera) indicate an alternation of different properties in the chromomeres and the connecting strands (129, 192, 27). The most plausible interpretation at present seems to be that the recognizable genes are relatively unbreakable physical and physiological units, probably associated with the chromomeres and bound together by material that is relatively inert physiologically, relatively easily broken and perhaps not even genic material (in the sense of capacity for duplication).

We have referred to attempts to estimate the number of genes. In Drosophila most of the mutations that are observed now turn out to be at least alleles of previously observed mutations. This indicates that the number of loci is not indefinitely great. Estimates based on frequencies of recurrence rest, however, on the hypothesis, known to be incorrect, that all loci mutate equally frequently. Estimates can also be made on the minimum distances between genes in parts of the chromosome in which rates of crossing over are typical, considered in

relation to the total length of the chromosome. Thus crossover frequencies of 0.1 per cent in a typical region of a chromosome about 100 units long would indicate a minimum of 1000 loci in that chromosome. Finally, is the evidence that genes are associated with the chromomeres. The indications are that there are at least several thousand genes in Drosophila though probably less than 10,000 (173, 176, 181, 109, 54, 27, 28, 255). Belling (17) estimated the number of chromomeres in the lily at about 2000. Lindegren and Rumann (153) put the number in Neurospora at less than 300.

Maximum estimates of the size of genes have been based on estimates of the volumes of the chromosomes, divided by minimum estimates of numbers of genes. These lead to maximum estimates (in Drosophila) of about 10^4 $(m\mu)^3$, approximately the same as for the particles of tobacco mosaic virus and much larger than hemoglobin molecules (about 1 $(m\mu)^3$). It is reasonably certain, however, that the genes constitute only a small fraction of the size of the visible chromosome. A gene may be no larger than a large protein molecule.

The chemistry of the chromosomes and allied questions have recently been reviewed by Gulick (111). Most of the material extractable from sperm heads (consisting largely of chromatin) is thymonucleic acid (about 60 per cent), combined with proteins of a very simple sort (histones and protamines (about 35 per cent)) (142). The characteristic staining reaction of chromatin to basic dyes is a somewhat nonspecific indicator of nucleic acid. The Feulgen reaction is more specific for the pentose component. Caspersson's (37) studies of the absorption spectrum of the salivary glands of Drosophila indicate that nucleic acid (with a strong band at about 2600Å) is in excess in the bands which stain dark in cytologic preparations. Digestion with nuclease removes this material but does not destroy the continuity of the chromosome (168). This is destroyed by digestion in trypsin (37, 168) but not in pepsin (168) in harmony with the hypothesis that continuity resides in the protein constituent and particularly in protamines and histones (not dissolved by pepsin). It is interesting that the crystalline viruses (230) which behave as genic material in the sense that particles are able to bring about the synthesis of more of their own kind of material in living protoplasm (tobacco virus in either tobacco or tomato cells) are nucleoproteins (5).

Nucleic acid is itself too simple a material and too uniform in nature to be responsible for the specificity of the genes. Similar statements have been made with respect to the protamines and histones extracted

from sperms. The possibilities of diversity among protein molecules through the different possible arrangements of the amino acids and through attachment of prosthetic groups is, however, so nearly infinite that there seems to be no theoretical difficulty in connection with gene specificity, even if only a minute portion of the visible chromosome is genic.

Gene Duplication. The most essential property of a gene is, of course, the capacity to bring about the production of an exact duplicate, irrespective, within limits, of associated genes or environmental conditions. The growth and fission of a cell present a certain analogy, but as cells are wholes of which the genes are components, it is to be expected that an understanding of cellular growth and reproduction must be based on gene duplication rather than the reverse. Consideration of the maximum possible size of genes and their regularity of duplication leads in the direction of an interpretation in terms of molecular mechanisms and hence to an analogy with crystal growth (141, 1, 176). There is the difference, however, that duplication must be associated with a mechanism of separation.

Among crystals the closest analogy is with the nucleo-proteins responsible for virus infections. As already noted these are of the same order of size as genes and have the same property of autosynthesis. The similarity of genes and viruses in these respects was indeed pointed out (67) before the discovery of the crystalline nature of the latter. More recently it has been shown (108) that viruses resemble genes in inactivation under x-ray treatment and that viruses may acquire new specificities (after x-radiation) analogous to mutations of genes (163).

The autosynthesis of a molecule of the degree of specificity of a protein seems to require, as often noted, that the original molecule behave as a model on which the duplicate is built up from the simpler substances in the medium. This requires attraction of like by like (cf. 182). The essential pattern can hardly be more than two dimensional to permit such duplication to be followed by separation. Such a scheme obviously provides for duplication as of the new type after mutation.

The pairing of homologous chromosomes at zygotene of meiosis and in the salivary gland cells of diptera demonstrates that there actually is attraction of like for like in cells under certain conditions. This attraction moreover has its seat in the chromomeres rather than in the chromosomes as wholes, since the pairing of homologous chromomeres occurs in cells heterozygous with respect to translocations, inversions, etc., even though the chromosomes must be thrown into crosses, loops,

etc., in order that this may occur (16, 161, 192). The suggestion that the postulated process in which genes attract an array of substances from the medium to form a duplicate is physically allied to the visible attraction between homologous chromomeres has often been made.

One of the most important lines of investigation on the nature of genes is the study of the occasional failure of exact persistence or duplication. There is an extensive literature on mutation which has frequently been reviewed (244, 124, 179, 188, 245, 218, 247). Considerations of space forbid such a review here. It may be noted, however, that the conception of the gene that seems to fit best the facts of mutation is that of a molecular pattern which may be replicated but not many times, usually highly stable, but with a threshold for internal rearrangement or other change that is easily surpassed on absorption of energy from a single excitation or ionization by x-rays, γ-rays, neutrons, etc., and occasionally surpassed by extreme thermal agitation (248).

Control of Genes by Cellular Physiology. While genes behave as autonomous entities in their capacity for duplication and in the incapacity of the cell to replace ones which have been lost or inactivated, in other respects they behave as components of an integrated system. For example, all chromosomes and hence presumably all genes duplicate as a rule just once in the cycle of cell division. It would exceed the scope of the present paper to discuss the processes of mitosis and meiosis and their implications for gene physiology.

It should be added, however, that while in these processes the genes behave as if subject to the physiological conditions of the cell as a whole, there is evidence that this physiology is in turn regulated by particular genes. A considerable number of genes are known which affect the regularity of mitosis and meiosis (106, 26, 240, 6, 7, 8, 9, 73).

Non-mendelian Heredity. The question as to how far hereditary properties are restricted to the chromosomal genes is one that must be considered briefly (reviews 79, 99, 228). In the first place, it must be noted that there are two groups of organisms—bacteria and blue green algae—in which the occurrence of a definite nucleus, of chromosomes, or of any sort of mitosis is usually denied (cf. however (152)). The existence of groups in which all heredity must apparently be non-mendelian suggests the likelihood of some heredity of this sort in higher organisms. Evidence of such heredity was indeed discovered early by Correns (44, 45).

The usual criterion for distinguishing non-mendelian heredity from mendelian is based on the great disparity in the amounts of cytoplasm

contributed by egg and sperm in contrast with the essential similarity of the nuclei. Thus a difference between reciprocal crosses which persists at least to F_2 suggests transmission in the cytoplasm unless it can be associated with a recognized nuclear difference (such as the distribution of the X and Y chromosomes). A difference, manifested in F_1 (and not due to sex linkage) implies dependence on a quality of the egg cytoplasm but not necessarily that this quality is carried autonomously by the cytoplasm from generation to generation. It may have been imposed on the cytoplasm, either by the egg nucleus before maturation or by absorption of substances from the mother. Several cases are known in which a character is mendelian but as an indicator of the genotype of the mother, presumably through one of those mechanisms: serosa color of the silk worm embryo (251, 242), lipochrome coloration of eye and body in newly emerged Gammarus (224, 225), ocellus color of the larva of the moth Ephestia (experimentally shown to be affected by the maternal hemolymph (35, 36, 145, 146), localization of extra bristles in the mutation polychaeta of Drosophila funebris (246), viability and fertility in several cases in Drosophila (205) and direction of coiling in the snail, Lymnaea (24, 238, 60).

There seem to be no wholly clear demonstrations in animals of non-mendelian transmission of *individual* differences through the germ cells. Kühn (144) found a slight but clearly transmissible difference between reciprocals on crossing strains of the wasp Habrobracon selected for difference in pigmentation. It is not clear, however, that the parent strains had been made isogenic before the cross. Jollos (137), working with protozoa, has found transmission for a limited number of generations of effects of treatment with chemicals, high temperatures, etc., that suggest cytoplasmic heredity. The same author (138) has described similarly behaving "Dauermodifikationen" as a result of temperature shock in Drosophila. This has been partially confirmed by Plough and Ives (201), who described a tendency toward sporadic appearance of abnormalities of development after such treatment, for several generations, exclusively down the female line.

Goldschmidt (98, 99, 100) has demonstrated cytoplasmic heredity of a number of differences between geographical *races* of Lymantria dispar (cf. however (264)). These include differences in the strength of the tendency to develop in the female direction, in rate of larval development and in larval pattern.

For characters that develop late in ontogeny a difference between reciprocal crosses (if not due to sex linkage), observed only in F_1, raises

a presumption of cytoplasmic heredity. There is evidence of this sort from Triton hybrids (121, 122) and merogones (3, 112, 113).

Clear cases of non-mendelian heredity are much more numerous in plants. Most of the cases of individual differences shown to be of this sort are in chlorophyl characters, especially chlorophyl variegations (cases in about 50 species listed by de Haan, (51)). The plastids divide and are transmitted in the embryo sac, rarely in the pollen. It is likely that these are cases of direct transmission of a plastid quality. It should be noted, however, that the plastids are far from being completely autonomous bodies. de Haan lists 73 chlorophyl characters in the corn plant as due to nuclear genes and only 3 as transmitted in a non-mendelian fashion.

The most exhaustively studied case of a non-mendelian individual difference not related to chlorophyl is one of pollen sterility in corn (210).

It is doubtful whether a sharp line can be drawn between non-mendelian heredity and virus infection. A case in Datura stramonium illustrates the difficulty (23). A type called quercina differed from normal in its leaf shape, low vigor and usual pollen sterility. It followed an irregular, apparently non-mendelian course of heredity. At first disease seemed ruled out by failure of transmission on rubbing leaves, or inoculating juice, but it was later found to be transmissible from affected to normal tissue after grafting.

A rather large number of cases have been described in which crosses between different subspecies or species of plants have given persistent differences in reciprocal crosses, where no question of infection in the ordinary sense is involved. Renner (208, 209) for example has shown that plastids whose straight female line of descent is from Oenothera Lamarckiana do not produce normal green pigment in any plants in which they come to be associated with nuclei of certain constitutions (e.g., homozygous Oenothera hookeri). Sirks (227) made crosses between Vicia faba major and V. faba minor. With major as the female parent, a block of linked genes behaved normally in F_2 (no differential viability, normal crossing over). In the reciprocal cross, the F_1 individuals exhibited effects of the genes of this block derived from the pollen plant (major), but produced no crossover gametes and in F_2 no homozygotes for the major chromosome appeared. The results must apparently be attributed to an interaction between the major chromosome and a transmissible property of the minor cytoplasm. Sirks also found differences in stem length and fruit length that were non-mendelian. Wettstein (258, 259) has described crosses between mosses

showing maternal inheritance of morphological characters for many generations. These were more pronounced in intergenoric crosses than in interspecific ones and were not observed in interracial crosses. Non-mendelian results from crosses between species of Epilobium have been studied by several investigators (reviews 79, 99, 228). Michaelis found that effects of disharmony between maternal cytoplasm and paternal genes on corolla length and pollen fertility were not removed by 13 generations of backcrossing. Pollen sterility is a common consequence of species crosses and has been shown in a number of other cases to be transmitted as if dependent on a persistent disharmony of the cytoplasm with the nucleus of the pollen parent.

Thus there can be no doubt that there are qualities of the cytoplasm that are autonomous. The astonishing thing is that cytoplasmic heredity plays such a small rôle. The general principle that reciprocal hybrids are closely similar if not identical was established by the early plant hybridists—Kölreuter, Gärtner, etc. Gärtner (quoted by Mendel, 169) demonstrated in 30 cases that hybrids could be transformed into a type indistinguishable from the pollen parent by 3 to 6 successive pollinations to the latter, indicating that the entire species heredity was transmissible by the pollen. Goodspeed and Clausen (103) obtained some apparently pure Nicotiana sylvestris from tabacum x sylvestris hybrids after only two such pollinations to the latter. In several cases in plants, merogones have been reported as developing into plants of purely paternal type (cf. 79).

The Genetics of Individual and Species Specificity. The usual subject matter of studies of heredity is found in the morphological and physiological characteristics of species and of individuals within species. In most of these cases, however, the relation between gene and observed character must of necessity be rather indirect. It is desirable to consider the most direct indicators of the specificity of different protoplasms and these seem to be provided by serological properties, including the reactions to transplantation.

Leo Loeb (158), working with guinea pigs and mice, demonstrated that the reaction to transplanted tissues (normal or tumor) varies with relationship. Autotransplants are readily accepted. Transplants from close relatives encounter a more or less hostile reaction and are usually rejected in the end. The reaction is more severe toward tissues from random animals of the same species and still more severe toward tissues from a foreign species. Little and associates (157, 154, 156) demonstrated that susceptibility or resistance to tumor transplants depends on

multiple mendelian genes. These genes do not, however, determine susceptibility or resistance as such, but a certain specificity, the reaction depending wholly, or almost wholly, on the presence of dominant specificity genes in the graft lacking in the host. The presence of such genes in the host, lacking in the graft, are of little or no importance. Thus in a typical experiment, all individuals of F_1 from a cross between different inbred strains are found to accept transplants from either parent strain. On the other hand, both parent strains reject transplants from F_1. The great majority of F_2 are usually resistant to a tumor from one of the pure strains. The proportion of susceptible could be interpreted as $(3/4)^n$ where n is the number of dominant specificity genes peculiar to the strain in question. By further breeding it was found possible to develop strains giving simple mendelian ratios (cf. 235, 21). Similar results have been obtained with leukemia in mice (162, 212) and with normal tissues in mice (155) and in guinea pigs (160). Results from inbred strains of rats, on the other hand, could only be harmonized on the hypothesis that these strains had not reached the same degree of homozygosis in the pertinent factors as in the other cases (159).

An especially important case is that of the blood groups discovered in man by Landsteiner (review, 262). The major groups (O, A, B, AB) have been shown to depend on a series of 3 multiple alleles, of which two (I^A, I^B) determine the presence of the specific isoagglutinogens A and B, while the third is recessive to the others and seems to determine nothing positive, at least in relation to A and B (group O = ii, group A = I^AI^A or I^Ai, group B = I^BI^B or I^Bi, while group AB = I^AI^B) (20). Cells from an individual with either A or B agglutinate in serum from an individual lacking the corresponding gene in analogy with the results from transplants. More recently it has been shown that A consists of two closely similar agglutinogens A_1 and A_2, determined by different alleles.

An independent pair of agglutinogens M and N, requiring absorbed serum from sensitized rabbits for demonstration, has been shown to be determined by a pair of alleles (148). There is no recessive in this series corresponding to i.

A number of such blood groups have been demonstrated in other mammals. Antigens serologically identical with A and B have been found in anthropoid apes. A series of 4 blood groups in rabbits is determined by 3 alleles of which one was recessive to the other two, parallel to the case in man (39, 40). These cases in mammals are

interesting as showing that specificity of the cytoplasm of anucleate cells may be determined by mendelian genes which must have acted before the nucleus was lost. The allelism of genes determining 2 or more antigens which differ positively from each other (as A and B) indicates a direct relation between the specificities of gene and antigen.

Specificity differences in the red blood cells have also been demonstrated in birds. Todd (249, 250) has shown that, in the domestic fowl, individuals can nearly always be distinguished serologically, even among close relatives. A polyvalent serum, capable of agglutinating the corpuscles of any fowl, would usually agglutinate the cells of a chick after absorption by cells of one of the parents but never after absorption by cells of both parents. The results imply that these differences are entirely dependent on multiple mendelian genes.

Especially instructive are the results of Irwin and associates (133, 134, 135, 131, 132) on 4 allied species of birds, the domestic pigeon (Columba livia), a wild pigeon (C. guinea), the ring dove (Streptopelia risoria) and the pearl neck (Spilopelia chinensis). Crosses and backcrosses were available between ring dove and pearl neck, between livia and guinea (reciprocal crosses) and between livia and ring dove, a family cross. The technique used involved production of antiserum from rabbits injected with red blood corpuscles and absorption of this by corpuscles from one or more sources, to produce reagents containing antibodies for the antigens present in the injected corpuscles but not present in the absorbing corpuscles. Each of the 4 species contained antigens lacking in all of the other 3. Other antigens were demonstrated to be common to all 4 species but some were demonstrated to be common to only two or three. The pearl neck was demonstrated by repeated backcrossing to have at least 10 independent dominant antigens lacking in the ring dove and at least 7 of these were isolated to a point at which 1:1 ratios were demonstrated by adequate numbers in the backcrosses. Similarly C. guinea was shown to have at least 6 independent dominant antigens not present in the tame pigeon. The absence of any demonstrable difference due to cytoplasmic heredity was brought out by a study of reciprocal crosses.

One of the most important results was the demonstration that the F_1 hybrids between ring dove and pearl neck and those between ring dove and livia do not contain all of the antigens of the parent species but in place of these produce serologically distinct hybrid substances.

A phenomenon of the plant kingdom that has been interpreted as dependent on biochemical specificity is self incompatibility (77, 78).

The commonest genetic mechanism involves a single series of multiple alleles. Pollen tubes with a given allele (e.g., S_1) in the haploid nuclei grow too slowly on a style whose cells carry the same allele (e.g., S_1S_2, S_1S_3, S_1S_4 $- -$) to reach the embryo sac, while there is no inhibition on styles with wholly different alleles (S_2S_3, S_2S_4, etc.). At least 15 such alleles have been described in Nicotiana alata and N. Sanderae (77) and 37 in Oenothera organensis (83). The specificity differences in fungi required for zygospore formation probably also belong in this category (22, 123).

These cases indicate that protoplasmic specificity is determined simply and directly by genes. A noteworthy point is that, in these higher animals and plants, the evidence indicates that substantially the entire difference in specificity between individuals and species is under control of mendelian genes.

The Relation of Genes to Growth. Growth in the sense of increase in the amount of active protoplasm is characterized especially by increase in protein. It has often been pointed out that the number of possible arrangements in even a small protein molecule is practically infinite (cf. 105). The only mechanism by which a given organism can produce particular ones in the array of possible proteins would seem to be autosynthesis by a preëxistent molecule acting as a model. This is the same mechanism that seems required for duplication of genes and suggests the hypothesis that each protein molecule is formed on a model carried in a chromosome (141). This, however, has obvious difficulties. It implies that growth proceeds linearly from minute components of the cell. Moreover the proteins emanating from the nucleus would have to pass through the nuclear membrane to permit cell growth between mitoses. There is abundant indirect and some direct evidence that gene products can pass through the nuclear membrane (review 234). Haemmerling, for example, has demonstrated control of form determining properties of the cytoplasm by the nucleus, by means of regeneration and grafting experiments with species of the giant one-celled alga Acetabularia (116, 117). Such passage seems hardly likely, however, for substances of such high molecular weight as the proteins.

An alternative view is that duplicates or partial duplicates of genes reach the cytoplasm when the nuclear membrane disappears in mitosis and that these can produce duplicates in turn, and so on, permitting exponential increase. But such particles, having the essential property of genes, would give practically pure cytoplasmic heredity unless it be supposed that their genic property is subject to decay. As noted, it

has been shown in some cases that there are cytoplasmic properties transmitted without apparent decay through many generations. These may reside in cytoplasmic proteins which possess the essential genic property (plasmagenes). Unless it is demonstrated that there is ultimate replacement by substances of nuclear origin, it is superfluous to trace them at all to nuclear genes (chromogenes).

There is the difficulty here that if the main constituent of the cytoplasm is autonomous one would expect more abundant evidence of cytoplasmic heredity than is found, particularly in connection with specificity. If the capacity of a protein to produce specific antibodies depends on its structure as a whole it would seem a necessary conclusion that the heredity of specificity is the heredity of protein structure.

It has been shown by Landsteiner (147), however, that the major portion of the specificity of the antibodies produced by a compound protein may be due to relatively simple chemical groupings (haptens), although the latter are unable to produce antibodies by themselves (review, 105). His results indicate that antibodies induced by the combination of a protein with a hapten react specifically with the hapten, either free, or in other combinations, and not to any great extent with the uncombined proteins. It is possible that specificity effects due to haptens whose presence is controlled by particular nuclear genes (chromogenes) overshadow specificity due to protein plasmagenes.

On the physiological side it appears that the presence of a nucleus is necessary for cell growth, although not for cleavage (126). This is not a mere matter of size of the nucleus in relation to cytoplasm. Even small deficiencies are usually lethal in the egg in Drosophila (151). Moreover, it has been shown that the great majority of small deficiencies in the X chromosome of Drosophila are lethal when homozygous, in single epidermal cells in an otherwise normal individual (53, 54, 55). In the presence of the genes of the type known as Minute, crossing over occasionally occurs in somatic cells (233, 139). If the two X chromosomes are properly marked, this crossing over results in recognizable sister spots provided neither chromosome contains a lethal. A cell lethal is demonstrated by the occurrence of an unpaired spot.

It may be concluded that, while the proteins of the cytoplasm are probably autonomous with respect to basic structure, metabolic processes are dependent on active substances, probably of relatively low molecular weight, emanating from the nucleus, and that the specificity of the proteins as antigens largely depends on such substances in combination with them.

Heredity in Relation to Differentiation. So far we have treated heredity as manifested in persistence of qualities from generation to generation. In the course of development of any of the higher organisms, the descendants of a single egg come to be exceedingly different along diverse cell lineages. These differences are not due merely to the immediate reactions of essentially similar protoplasms to different local conditions since they persist and are handed on during cell multiplication in a neutral environment. Tumor cells derived from a particular tissue may be transplanted to foreign positions in a long succession of hosts without losing their tissue specificity. Differentiated cells grown in tissue culture may lose more or less of their morphological differentiation but in general may be shown by suitable experiment to retain their physiological differentiation (review 130). This persistence of properties can only be interpreted as involving cell heredity. There are various possible genetic hypotheses.

Weismann postulated a differential sorting out of nuclear determiners in all cell lineages, except those of actual or potential germ plasm. This view does not appear tenable in view of the equational character of mitosis. Every cell receives a full length replica of every one of the chromosomes including, for example, such specialized cells as those of the salivary glands and midgut of diptera (19).

Another possibility is that certain genes are transmitted in an inactivated condition and that irreversible activation is induced systematically under special local conditions. The result would be a developing pattern of heredities related to the spatial organization of the embryo. There is a class of highly mutable genes responsible for certain types of variegation. In certain cases, somatic mutation of this sort is under some degree of local control. Thus in Drosophila virilis (52) the recessive, miniature α, mutates freely in both germ line and soma. It gives rise to a mutation, miniature γ, in which mutation is all but completely suppressed in the germ line. In the presence of certain other genes, mutability in the soma is, however, increased to a point at which all flies are mosaic. If the mutability of a gene of this sort should come under such complete control of local conditions as to mutate in an orderly pattern in the soma, without ever mutating in the germ line, it would obviously give a basis for permanent differentiation in cell heredities.

The usual and most probable view is that cellular differentiation is cytoplasmic and must therefore persist and be transmitted to daughter cells by cytoplasmic heredity. The chief objection is that it ascribes enormous importance in cell lineages to a process which is only rarely responsible for differences between germ cells, at least within a species.

It may be, however, that the more or less complete early isolation of the germ line of higher organisms has come about in evolution to maintain a line of cells with plasmagenes lacking in prosthetic groups and hence in specialized activity but capable in somatic cells of combining with such groups emanating from the nucleus to form molecules that multiply thereafter as plasmagenes of a more specialized sort. Under this viewpoint, differences in the local conditions may bring about differential accumulation of metabolic products arising from the interaction of cytoplasm with nuclear products and environment, and eventually lead to the elaboration of new plasmagenes in the cytoplasm in particular regions of the organism. In fact, in eggs of the less regulatory sort, irrevocable differentiation occurs in certain regions of the cytoplasm before cleavage and so cannot be a consequence of nuclear differentiation of any sort. As development proceeds, each step in the regional differentiation of cytoplasmic heredity increases the diversity of local environments and so facilitates further differentiation.

Genic Control of Enzyme Differences. There is a certain similarity in all cells in gross chemical constitution and physical organization. Yet there is the greatest diversity in the substances produced. The usual interpretation is that there is an almost infinite field of possible syntheses of which protoplasm is capable, but that in each case the course of metabolism is guided along particular paths by the particular assemblage of specific catalysts. The demonstration that several enzymes are proteins capable of repeated crystallization without loss of properties has suggested that their specificity rests on the same basis as that of other proteins or protein compounds. The mode of inheritance of enzyme differences is obviously a question of great importance in physiological genetics (cf. 253, 97, 174, 32, 257).

Differences in pigmentation are among the characters of vertebrates whose genetics have been studied most intensively. The melanin pigments are produced in specialized cells which have been shown in several cases (75, 76, 62, 63, 80) to migrate from the neural crest. In certain spotting patterns, it is probable that these cells fail to reach all parts of the skin (cf. 43). In other cases, however, it is clear that the differences in color depend directly on differences in cell physiology. It has been shown from somatic mutations (274, 70, 200) and transplantation (47, 48, 49, 206, 207) that these differences depend largely on genes in the cells themselves rather than on hormones elaborated elsewhere, although the possibility of control by hormones is illustrated by the well known effects of ovarian hormone on plumage color in the fowl.

The melanin pigments are produced from tyrosin or allied substances

(especially dopa) under the influence of oxidases. Cuénot (46), as early as 1903, suggested that genes might control color differences by control of enzymes. Several authors have reported differences in tyrosinase or dopa oxidase content of extracts from skins of laboratory mammals of different genotypes (74, 189, 140, 42). The results were variable and apparently involved factors not under complete control (cf. 204). The situation seems to have been cleared up by the work of Danneel and Schaumann (50) who devised means of separating dopa oxidase from inhibitory substances normally present in skin extracts. They have given evidence for a chain of 3 processes: 1, a process suppressed by x-rays; 2, anoxidative formation of dopa oxidase, occurring in black rabbits (genotype C-) even under continuous high temperature (37°) but only after a period of exposure to temperatures below 33° in Himalayan rabbits, whites which develop black in exposed parts of the skin (219, 221) (genotype c^hc^h), and failing completely in true albinos (genotype cc); 3, a reaction resulting in pigment formation, requiring oxygen and inhibited by HCN.

Another method of demonstrating the occurrence of dopa oxidase has been the reaction of pigment cells in frozen sections of skin to buffered solutions of dopa. Differences have been found between different genotypes of the rabbit (220, 143) and the guinea pig (143, 214).

There are mendelian differences in the melanic pigmentation of the beetle, Tenebrio mollitor. Schuurmann (222) found a corresponding difference in ferments from the hemolymph but no demonstrable difference between chromogens extracted from the exoskeleton. The ferments from two genotypes produced different pigments not only with extracts of the natural chromogen but also with pyrocatechin.

Matsumura (166, 167) has described differences between races of silk worms in amylase activity of the digestive juices and of the body fluids as due to two strongly linked pairs of alleles. Strong activity was dominant over weak in both cases.

White color of the fat in rabbits differs from yellow by a single dominant gene (197). The yellow color of the recessive fails if the rabbits are not fed green leaves. It appears that a xanthophyllase is lacking in the recessive.

Dalmatian hounds excrete more nitrogen as uric acid (2 to 3 per cent) than other breeds (0.2 to 0.4 per cent). Low uric acid excretion is dominant over high (190, 252).

Hyper- and hypoglycemia in mice are both recessive to normal (33, 34) and apparently belong to the same allelic series with high blood sugar dominant over low (69).

Alkaptonuria in man has long been known to be a simple recessive condition (96). Alkapton is not broken down in the recessives, presumably because of lack of the appropriate enzyme. The situation appears to be similar (96, 119) with respect to a number of other hereditary errors of metabolism in man, e.g., steatorrhea, hematoporphyria, pentosuria and cystinuria.

A type of hereditary feeblemindedness is associated with excretion of phenylpyruvic acid. This condition appears to be inherited as a simple recessive (198, 199).

There are numerous metabolic differences in plants that depend on genes. An especially interesting case is that of a recessive gene in corn which changes the nature of the starch in the endosperm, pollen grain and embryo sac. Brink found that the effect in the pollen grain, only 2 cell generations from the reduction division, depended on a difference in amylase activity (31).

Gene Control of Chain Reactions. Breeding experiments appear rather crude as a method of physiological investigation. Yet from one viewpoint they constitute a rather delicate method, especially if supplemented by study of the physiological and biochemical nature of the character differences. One can make exactly one or two or (in polyploids) more replacements of one of the ultimate physiological units of living protoplasm without experimental disturbance. The quantitative effect of a given gene replacement can often be studied in a great diversity of genetic as well as environmental conditions.

There has been considerable study of the coat colors of mammals from this viewpoint (267, 268, 269, 273, 72), although there is still the disadvantage that the chemical constitution of the melanins has not been worked out. The colors fall into two main series, dark (or melanic) and yellow (or xanthic). Within the dark series it is probable that at least the sepias and browns differ qualitatively. Something of the complexity of the system of interactions may be seen from table 1 which gives the relative quantities of pigment found (by colorimetry or titration with $KMnO_4$) in guinea pigs in combinations of 5 series of alleles (273, 213, 128).

Inspection of the table shows that the various genes do not determine the colors in any simple way. The sepias and browns differ from most of the yellows by the presence of E. There are, however, two ways of getting yellow in the hair in spite of the presence of E. In the combination EA (not shown) there is a yellow subterminal band in each hair, of the same intensity as in the corresponding combination with ee. In Effpp the hair is clear yellow. if colored at all, but a much paler yellow

than with eeffpp. In the former case, it appears that the melanic process is blocked *before* it has interfered with yellow, in the latter case, *after*.

The C series affects the intensity of whatever color is determined by the other genes (C intense, $c^a c^a$ white) but the effects are far from parallel. Only 3 alleles can be disinguished confidently in effect on

TABLE 1

The average concentrations of pigment in the hair of guinea pigs at birth

The sepias and browns are given on a scale in which intense black (EPBC) is 100. The yellows are given on a scale in which intense yellow (eFC) is 100. The latter actually has only about 20 per cent as much capacity for reduction of $KMnO_4$ as intense black. Replacement of a, assumed above, by A, replaces the sepia, brown or yellow of E-combinations by the yellow of the corresponding e-combinations in a subterminal band in dark hair. There is no effect in e-combinations. The recessives are here represented by single symbols except in the C— series.

	SEPIA		BROWN		YELLOW			EYE COLOR (E, e; F, f WITHOUT EFFECT)		
	EFPB EfPB	EFpB	EFPb EfPb	EFpb	EfpB Efpb	eFPB eFPb eFpB eFpb	efPB efPb efpB efpb	PB	Pb	pB pb
C—	100	21	50	17	6	100	36	Black	Brown	Pink
$c^k c^k$	90	18	42	15	0	38	5	Black	Brown	Pink
$c^k c^d$	82	15	47	13	0	41	5	Black	Brown	Pink
$c^d c^d$	64	9	40	11	0	38	5	Black	Brown	Pink
$c^k c^r$	94	14	43	13	0	19	0^+	Black	Brown	Pink
$c^k c^a$	73	9	37	11	0	18	0^+	Black	Brown	Pink
$c^d c^r$	75	7	44	7	0	14	0^+	Black	Brown	Pink
$c^d c^a$	40	5	31	6	0	14	0^+	Black	Brown	Pink
$c^r c^r$	84	5	45	6	0	0	0	Dark red	Dark brown red	Pink
$c^r c^a$	46	1	33	1	0	0	0	Light red	Light brown red	Pink
$c^a c^a$	0	0	0	0	0	0	0	Pink	Pink	Pink

yellow (eeF) causing 4 levels (C 100 per cent, $c^{kd}c^{kd}$ 39 per cent, $c^{kd}c^{ra}$ 16 per cent, $c^{ra}c^{ra}$, white, using compound symbols (e.g., $c^{kd}c^{kd}$ includes the 3 genotypes $c^k c^k$, $c^k c^d$, and $c^d c^d$)). In sepias (EPB) these 4 levels are replaced by a series of *waves*—a drop in intensity of sepia accompanying each upward step in yellow except the last. There appear to be differences in *threshold* for sepia and yellow, and *competition* between the two

processes (267). The melanic pigment of the eye (which seems to lack yellow) does not agree with that of the coat (c^dc^a more intense than c^rc^r in eye but less intense in the coat).

Replacement of either P by pp or of B by bb greatly reduces melanic pigmentation in coat, eye and skin, but does not affect yellow. But in the former case, there is exaggerated dilution in the lower compounds of the C-series while in the latter there seems to be a ceiling effect, relatively little difference among compounds of the C-series except in albinism (c^ac^a) itself, a situation found also in the mouse (72). The combination of bb with pp produces little if any greater dilution than in Bpp, although there is a somewhat browner quality found in all comparisons of browns with sepias.

Replacement of F by ff greatly reduces yellow. The effect in the lower compounds of the C-series is exaggerated from the standpoint of percentage effect. In $c^{kd}c^{ra}$ all yellow is lost except for occasional traces. Albinism in these combinations behaves as if almost completely dominant over c^k and c^d. This replacement (F by ff) has no effect on sepias and browns that carry P but in association with pp all melanic pigment is absent leaving pale yellow or white.

The genes C, E, P and B appear to be completely dominant over their alleles but in the case of F, dominance is not complete, eeFfC having about 94 per cent as much yellow as eeFFC. There is incomplete dominance of various degrees among the lower alleles of the C-series.

Yellow pigment is almost wholly displaced (not merely concealed) by the melanic pigments in most cases where these are present, indicating another sort of competitive effect from that referred to above. In the case of c^dc^a combined with EFppB, however, there is so little sepia that there is often a close approach to pure pale yellow in contrast with the slate color of c^kc^a and c^rc^r in the same combination.

Additional criteria of the modes of action of these genes are given by the dopa reaction (214). That in the hair follicles must be distinguished from that in the basal layer of the epidermis as they do not run parallel. The reaction in both cases is almost as great in yellows (ee) as in the corresponding sepias or browns (E) and does not appear to be affected by the A series. In the hair follicles the compounds of the C-series give reactions closely paralleling in intensity (not in color) the effects on yellow, whether the hair is melanic or xanthic. Replacement of F by ff reduces the intensity of the reaction, while replacement of P by pp or of B by bb has little or no effect. There is an indication here that the dopa reaction in the hair follicles is largely a test for an enzyme

concerned in yellow pigmentation, even in melanic individuals. The situation is very different in the basal layer of the same individuals. There is little or no reaction unless both C and P are present. Replacement of F by ff seems to have no effect. So far there is parallelism with the effects of these genes on melanic pigment, although a higher threshold in the C-series. Replacement of B by bb, however, seems to be without effect.

It is obvious that the effect of a gene replacement in one genetic background is usually very different from its effect in another. The genes cannot be supposed to be producing pigment independently of each other. The indications are that each acts at one or more definite places in a system of interacting chain reactions and that the effect of each is on the rate of the process which it affects. Figure 10 represents a simple system capable of accounting for these effects.

There has been a great deal of study of the interactions of the numerous mutations affecting eye color in Drosophila melanogaster. Morgan and Bridges (175, 25, 30) showed that while in some cases the combination of two mutations gave effects that appeared to be merely the sum of their separate effects this was by no means always the case. Where mutations resembled each other closely, the combination was also usually indistinguishable. In other cases, they found a disproportionately great effect in the combination, or even a reversal of the apparent direction of effect of one of the mutations. A number of specific modifiers were found, mutations that could be recognized by a modifying effect on another mutant although without apparent effect by themselves.

The relations between brown, scarlet and vermilion illustrate the deductions that are indicated in such cases (271). Scarlet (stst) and vermilion (vv) and the double recessive (ststvv) are all practically indistinguishable. These results suggest that st and v are inactivations of two genes (st^+, v^+), each of which is solely (or almost solely) responsible for carrying through a different link in the same chain reaction. The result is the same if either or both fail. The fact that the double recessive "scarlet-brown" (ststbwbw) is pure white suggests that bw is an inactivated phase of a gene (bw^+) solely responsible for a link in the chain of processes responsible for the pigment left in scarlet. If these deductions are correct both processes should also fail in the double recessive vermilion-brown. This turned out to be practically the case.

Schultz (216) and Mainx (164) found that the pigmentation processes of brown and scarlet not only differ qualitatively but are to some extent

segregated spatially. In scarlet the pigment consists of water soluble, yellowish red granules, largely located in the cells in the outer ends of the ommatidia. In brown the pigment was in highly insoluble, brownish red granules, surrounding the bases of the ommatidia. Mainx compared the effects of more than 20 mutations on these two processes by finding the combinations of each with scarlet and with brown. Two other genes, cinnabar and cardinal, closely resembled scarlet and vermilion and could also be interpreted as inactivations of normal genes determining links in the chain of processes leading to formation of the brownish red basal pigment. Most of the mutations, however, weakened both processes, although to widely different extents in different cases. Only one locus has been found in which the normal allele is so essential that complete inactivation prevents any pigmentation (white eye). Mainx found two loci (clot, sepia) which produced no apparent reduction in the quantity of either basal or distal pigment but instead modified the quality of the latter. He obtained extracts from sepia eyes which contained a ferment (absent in other genotypes) which would not only oxidize the distal pigment of sepia to an insoluble brown but do the same to the pigment of scarlet. This enzyme is interesting as occurring in a recessive mutation.

Another line of attack traces to Sturtevant's observation (236) that vermilion contrasts with other sex-linked mutations in not being autonomous in gynandromorphs. He interpreted this as evidence that the normal allele of vermilion produces a diffusible product.

Beadle and Ephrussi (12, 13, 84, 88) found that larval eye disks could be transplanted successfully to the abdomens of other larvae where they developed into slightly distorted eyes. Vermilion and also cinnabar eye rudiments implanted in larvae of red eyed (wild type) stock were found to develop into red eyes, although eyes from some two dozen other mutations developed autonomously in similar experiments. In the reciprocal experiment, the eyes of wild type larvae developed autonomously (red) in vermilion and cinnabar hosts and in all other mutant hosts (except claret where they became claret, an effect not yet cleared up). That the type genes, v^+ and cn^+, determine the production of a diffusible substance or substances, lacking in vermilion and cinnabar flies, was demonstrated by the induction of increased pigmentation in the nearly white eyes of vermilion brown and cinnabar brown by injecting (243) or feeding (14) the larvae with extracts of pupae from red eyed stock. Various experiments demonstrate further that two substances must be involved and that the production of the cn^+-hormone

depends on production of the v^+-hormone. Thus vermilion eye rudiments in a cinnabar host become red, indicating the presence of the v^+-hormone in the latter while cinnabar eye rudiments in a vermilion host merely become cinnabar, indicating lack of both substances in vermilion.

The similarity of vermilion and cinnabar (whose type alleles are involved in production of diffusible substances to scarlet and cardinal (whose type alleles act only locally) illustrates the point that diffusible and non-diffusible substances may enter into the same chain of gene controlled reactions (cf. 10).

The analysis of Drosophila eye color is by no means complete but the results already obtained nevertheless illustrate well the possibilities of combined genetic and physiological experimentation (cf. 85, 86, 87, 89, 90).

A similar situation has been found in the flour moth Ephestia kühniella in which a dominant gene A distinguishes a type with black eyes and pigment in brain and testis sheath from a type without this pigmentation (red eyed). Caspari (35) showed that implants of testes from dominants (AA) remain dark themselves and result in blackening of the eyes of aa hosts while aa testes in AA hosts become dark and do not affect the eyes of the host. The effect of the A-substance has been studied in much detail (146, 145).

Another case of this sort has been described in the parasitic wasp Habrobracon juglandii (260, 261). Males are haploid. Occasionally mosaic males appear whose characters are such as to indicate segregation at the first cleavage following failure of the second maturation division. Black-white (Wh-wh) and black-cantaloup (C-c) and black-red (Rd-rd) eye mosaics have a sharp line of cleavage between the colors, indicating the absence of any diffusion. This is not the case in black-orange (O-o) or black-ivory (O-o^i) mosaics. A beautiful natural experiment occurred in mosaic males of constitution cO-Co^i. The pale colors cantaloup and ivory were separated by a heavy black belt, sharply defined on the side toward cantaloup but fading through orange into the ivory.

Injection and feeding tests have now shown that the A substance of Ephestia is the same (as far as present tests go) as v^+ substance of Drosophila and that the O substance of Habrobracon shows a similar relation to cn^+ of Drosophila (11). These or similarly acting substances have indeed been found widely distributed among arthropods.

In the cases above, it appears probable that the diffusible substances are relatively directly related to gene action. Gene replacement appears

to affect merely the metabolism and not the morphology of the tissues producing them. There are other cases in which gene replacements make a difference in quantity or quality of a diffusible substance but in which this difference seems to be secondary to morphological effects. This seems to be the situation in the relation of the sex hormones to the X chromosomes of vertebrates (review 263), in the case of a recessive dwarf mouse due to a defective pituitary (229) and in that of the mutation, lethal giant, in Drosophila melanogaster in which delayed pupation is related to defect of the ring gland (114, 115, 215).

Analysis of the system of factors determining melanic pigmentation of the eyes in the amphipod, Gammarus chevreuxi, by Ford and Huxley (94, 95) is interesting from the fact that it was possible to measure the amount of pigmentation at all stages in the process in most of the combinations. The upper limit was reached very rapidly in the presence of gene R, but with rr (temperature constant at 23°) the amount of pigment with various combinations of modifiers (S-M-, S-mm, ssM-, ssmm) increased in sigmoid curves, at widely different rates at first but approaching limits which were all relatively close to the intense black of R although still preserving the same order as the initial rates. With different temperatures, but a constant genotype, a closely similar series of sigmoid curves was obtained. One gene (d) produced a wholly different effect, however. Pigmentation was almost absent until sexual maturity when it rose to the same level as with its allele D in the usual sigmoid course.

The physiology of factor interaction is more advanced in the case of flower colors than in any other case of comparable complexity since the chemical nature of the pigments has been worked out. Genetic studies from this viewpoint were begun by M. W. Onslow (191) and have been continued by numerous workers since (reviews 223, 149).

The colors depend in part on yellow and orange plastid pigments (carotin, xanthophyl) and in part on sap pigments. The two groups are not related chemically and are rarely affected by the same genes. The following discussion will be restricted to the sap pigments.

The anthoxanthins (flavones and flavanols) are responsible by themselves for sap colors ranging from ivory to intense yellow. The anthocyanins give colors ranging from scarlet through magenta and violet to pure blue. They are glycosides of anthocyanidins. The similarity and the essential difference between anthocyanidins and anthoxanthins is illustrated below in the cases of pelargonidin and apigenin respectively.

The yellow flavone luteolin differs from apigenin (ivory) in having

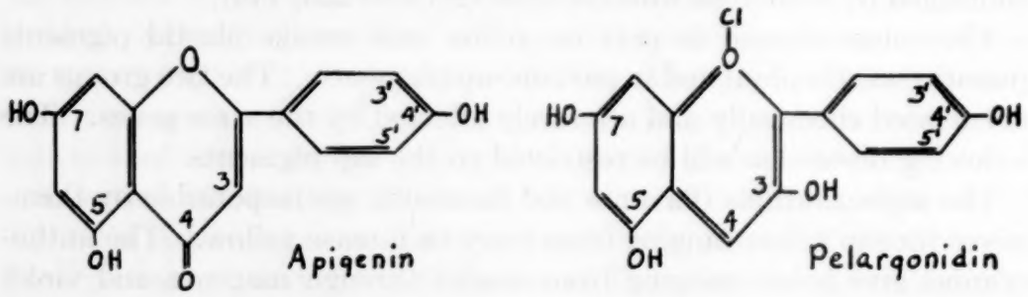
hydroxyls at both 3′ and 4′ on the side phenyl ring. The flavonols differ from the flavones in having the hydrogen at 3 replaced by hydroxyl.

In spite of the similarity of the anthoxanthins and anthocyanidins, there seems to be no such correlation in occurrence as would indicate derivation of one from the other. The evidence indicates rather derivation from a common source.

The anthocyanidins fall into three groups according to the number of OH (or OCH_3) groups on the phenyl ring. The simplest representatives of these are pelargonidin with one OH (at 4′), cyanidin with two (at 3′ and 4′) and delphinidin with three (at 3′, 4′ and 5′). One or more of the hydroxyls at 3′, 5′ or 7 may be methylated. The actual pigments (anthocyanins) are glycosides. Most of them have a monose (usually glucose, sometimes galactose or a pentose) at 3, a biose (i.e., disaccharide) at 3, or hexose molecules at both 3 and 5.

The color of the pigment depends on a variety of considerations all of which are subject to gene action. The more oxidized pigments are bluer than the less oxidized ones. Methylation tends to redden the color. The same is true of increased acidity. The 3-biosides are bluer than 3-monosides, and 3-5 dimonosides are still bluer. The presence of certain substances including some of the anthoxanthins (e.g., apigenin) tends to change the color of the anthocyanins in the blue direction by what is called co-pigmentation, probably due to weak additive complexes, the derivatives of delphinidin being most affected and those of pelargonidin least. Finally there seem to be special colloidal conditions in some cases, responsible for intense blueness.

An especially instructive analysis is that of Lawrence and Scott-Moncrieff (150) of the colors of Dahlia variabilis. As this is an alloоctoploid, it is possible to get each gene in any dosage from 0 to 4, making the



case unusually favorable for consideration of the quantitative effects of gene replacement. There are five main color factors. Gene I is necessary for production of the ivory flavone apigenin. Little or none is produced by one dose (I_1i_3) indicating a threshold, but deep ivory appears with two or more doses (I_2i_2 etc.). One dose of gene Y pro-

duces large quantities of an isomeric yellow flavone. No more is produced with higher dosages. It was shown that this yellow flavone not merely masks the ivory but tends to inhibit its production. An inhibiting factor H tends in turn to suppress the yellow flavone, giving primrose or cream colored flowers and permitting the ivory flavone to develop.

Gene A usually causes the appearance of anthocyanin. Increasing dosage produces increasing intensity leading to deep rosy magenta in $A_4b_4i_4y_4$. The pigment here is entirely cyanin. The addition of apigenin from increasing doses of I has two effects. The color is changed to bluish magenta by co-pigmentation and the intensity is reduced by a competition. Thus $A_1A_3b_4I_4y_4$, is pure ivory. This competition is interpreted as indicating that the two pigments trace to a limited common source.

Gene B is responsible for a much heavier production of anthocyanin than A. (a_4 to A_4)$B_1b_3i_4y_4$ is a deep crimson purple. The pigment is still all cyanin but with increasing dosages of B, pelargonin appears, shifting the color in the red direction (deep purplish crimson). The addition of apigenin (I_2i_2, I_3i, I_4) again shifts the color in the blue direction by co-pigmentation and again reduces intensity by competition. The tendency to replace cyanin by pelargonin increases.

The yellow flavone has no co-pigmentation effect on the anthocyanins, due either to A or B. It has, however, a more severe competitive effect than apigenin so that even such genotypes as $A_4b_4Y_4$ and $A_1A_3B_1b_3Y_4$ are pure yellow. Such anthocyanin as appears with Y present is wholly pelargonin in the absence of B($A_4b_4Y_1y_3$ deep apricot) unless the yellow flavone is largely inhibited by H, in which case there are mixtures of cyanin and pelargonin (magenta-apricot). It is to be noted that Y shows cumulative effects in combination with A($A_4b_4Y_1y_3$ deep apricot, $A_4b_4Y_3y$ tinged yellow, $A_4b_4Y_4$ clear yellow) although apparently completely dominant in the absence of A or B. A similar cumulative effect is shown in the presence of B. Thus $a_4B_1b_3Y_1y_3$ is scarlet but $a_4B_1b_3Y_3y_1$ tends to be orange and $a_4B_1b_3Y_4$ may be clear yellow.

It is to be noted that no detailed specificity is shown by the anthocyanin factors in this case. Either A or B may produce pure cyanin or pure pelargonin or a mixture depending on other factors. Increase of any pigment (apigenin and yellow flavone as well as anthocyanin) tends to decrease the state of oxidation of such anthocyanin pigment as appears, indicating another sort of competitive effect.

In the China aster (Calistemma chinensis) Wit (265) finds a series of general anthocyanin factors (W, maximum quantity; w^d, small quantity;

w, no anthocyanin whatever). The state of oxidation appears at first sight to be determined rather specifically by a series of three alleles. With rr (pink, red) there is pelargonidin glycoside (one OH on phenyl ring), with r′r′ or r′r (lilac, purple) this is replaced by the corresponding cyanidin glycoside (two OH's on the phenyl ring) and with RR, Rr′ or Rr (blue, violet) this is replaced by the corresponding delphinidin glycoside (three OH's on the phenyl ring). These anthocyanins are stated to be somewhat mixed, however, and when ii (assumed above) is replaced by I (incompletely dominant) the colors are darkened and made bluer by addition of cyanidin to pelargonidin in rr and of delphinidin to cyanidin in r′r′ and r′r. It appears that, after all, the R and I series produce a general tendency toward increased oxidation rather than specifically oxidizing particular chromogens.

Several other cases have been analyzed both genetically and chemically with similar results.

We have noted that there is usually a simple one to one relation between gene and the substances responsible for immunological specificity. The suggestion is that these substances or at least the active group (hapten) is a direct product of the gene, perhaps a partial duplicate, reflecting the specificity of the gene itself. A similarly direct relation has been suggested by Mangelsdorf and Fraps (165) in the case of a gene in corn that determines yellow color and vitamin A content in the triploid endosperm. The effect, measured by vitamin A content in feeding tests, was almost exactly proportional to the gene dosage, a relation confirmed by direct determination of the caratinoids (136). In general, however, the relation between gene and such substances as the animal and plant pigments and excretion products is less direct. Many genes, often acting apparently in sequence, are necessary for a particular product. In many cases a gene is more or less replaceable by other genes. Competition effects are common. In certain cases it has been demonstrated that the gene acts on the observed character by determining the presence of more or less specific enzymes. It is likely that the relation of the gene to these enzymes is in some cases of the same sort as the relation to antigens, but interpretation of the observed characters must in any case be in terms of reaction rates determined by such agents.

Theories of Dominance and Factor Interaction. Whatever theory is applied to the interactions between different series of alleles should also apply to the effects with a single series. This raises the problem of dominance, including the usual dominance of the normal type over mutations. The first theory was that of Bateson and Punnett (4) who

considered that the dominant member of a pair of alleles is a positive entity of which the recessive is merely the absence. They held that one dose of such an entity was likely to produce as much effect as two on the ground that the total effect might usually be expected to be limited by other conditions. This theory encountered difficulty with multiple alleles and with reverse mutation. If, however, it is supposed that the recessive represents inactivation of the gene in the respect in question, without loss of its essential structure, the latter difficulty disappears. Another theory is that of Fisher (91, 92, 93) that dominance is purely a matter of natural selection. The heterozygotes of unfavorable mutations are held to be selected for similarity to the type of the species until they come to resemble it completely through the fixation of specific modifiers of the heterozygotes. The adequacy of the theory in this form has been questioned (270, 272, 118, 120, 125). Plunkett (202) and Muller (178), however, suggested a selection process tending to build up a factor of safety for the type character in relation to factors of all sorts, environmental as well as genetic. This gives a broader basis for selection and there seems no doubt that such a factor of safety actually is the rule. Whatever the system of modifiers built up by natural selection, there must also, however, always be a complete physiological interpretation of dominance.

There is a certain amount of direct evidence for the inactivation theory of recessiveness. The effect of a deficiency usually resembles or exaggerates the effect of recessives whose locus is in the deficient region (171). This applies in the rare case of a deficiency that can be obtained homozygous (e.g., yellow achaete of Drosophila melanogaster (184)), as well as in the numerous cases of pseudodominance of recessives in heterozygous deficiencies. The dominant allele is usually dominant over deficiencies. These demonstrate that if either the dominant or recessive is inactive, it must be the latter. More satisfactory evidence is given in cases in which three or more representatives of a recessive gene can be introduced into a predominantly diploid zygote. If the recessive has a positive effect causing deviation from type, three should produce more deviation than two, but if it merely has a weaker effect of the same nature as that of the type allele, three recessives should approach closer than two to the effect of the latter. If it is completely inactive, three should have the same effect as two. It appears from such tests as have been made (231, 183, 178) that the completely recessive mutations are largely amorphs (no effect) or hypomorphs (weaker effect than type) in Muller's convenient terminology. Mutations exhibiting partial dominance over type are, on the other hand, usually

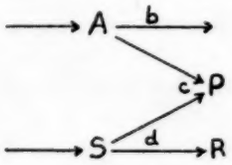
neomorphs (new type of effect) or antimorphs (inhibition of effect of type gene). A stronger effect than type Muller calls a hypermorph.

The majority of multiple allelic series have effects that fall at least approximately in a single linear series, usually with dominance of the highest member over all others, but incomplete dominance in lower compounds. Such series can be interpreted readily as ones in which all variations of the gene, whatever their nature, affect the strength of a single primary reaction (268, 272, 172, 180). Such alleles can be classified as amorphs, hypomorphs, type, and hypermorphs.

On the other hand, there can be no question now that there are many multiple allelic series in which alleles have qualitatively different effects. Examples have already been given in connection with serological specificity. Among other cases are the agouti series in mice (68), the black extension series in rabbits (38, 203), brachyury in mice (71, 73), certain colors of the pigeon (127), color blindness in man (254), scute, truncate, spineless and other series in Drosophila (64, 29), pericarp color in corn (2, 82), leaf shape in cotton (226) and perhaps certain pattern factors in grouse locusts (185, 186) and in the fish Platypoecilus (15, 104) if these are not due merely to close linkage.

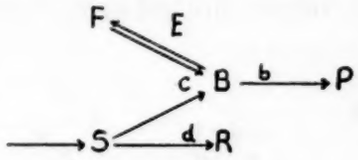
It is indeed probable that multiple changes at a locus are in general all qualitatively different with respect to the structure of the genes themselves and that this is manifested in the effects, if these are directly related to the constitution of the gene, as is probable in immunological properties. Whether manifested in later reactions or not depends, however, on whether the gene or its primary product enters into only one or more than one such reaction (268).

It is usually easy to suggest a plausible interpretation of the dominance relations or the factor interactions in any particular case, but it is difficult to appreciate the implications throughout an extensive network of reactions unless one introduces numbers. For processes such as pigmentation, that take place continuously for considerable periods of time, the simplest hypothesis is that of flux equilibrium for the duration of the process (272, 273). The relation between the rate of formation of a substrate, acted on by agents in various quantities or efficiencies, and the rate of formation of a product is what is needed in attempting to interpret chain reactions under this viewpoint. The rate of formation of the product, $\dot{P}$, depends jointly on the concentrations of the agent, (A), and of the substrate (S), i.e., $\dot{P} = c(A)(S)$. Under flux equilibrium these concentrations depend on the balance between the rates of production ($\dot{A}$ and $\dot{S}$ respectively) and rates of dissipation in the formation of the product (rate c(A)(S)), or otherwise (rates b(A) and d(S)).



$$\dot{A} - c(A)(S) - b(A) = 0$$
$$\dot{S} - c(A)(S) - d(S) = 0$$
$$\dot{P} = \frac{c}{bd}(\dot{S} - \dot{P})(\dot{A} - \dot{P})$$

If the agent is a catalyst, produced in certain quantity (E) and forming an intermediate product (B) with the substrate, the equation is the same as above or substituting b(E) for $\dot{A}$, where $\dot{P} = b(B) = c[(E) - (B)](S)$.



The quantity E may or may not be the same for all alleles. They may differ independently in the rate constants c and b. The products may differ in specificity in the same sense as the alleles. Thus products formed equally freely from the substrate (same c and b) may differ in efficiency in relation to the characters actually observed. There is no difficulty on this basis in understanding how certain alleles may be amorphs (c = 0), others hypomorphs, other hypermorphs in relation to type, and others antimorphs, the last transforming the substrate into a product of zero efficiency and thus destroying substrate that might otherwise be utilized. An allele that produces freely a product of low efficiency would behave as a hypomorph in heterozygotes with an amorph, but as an antimorph in heterozygotes with a more efficient allele. The term mixomorph has been suggested (273). A possible example is found in the genes for white (w), pearl (w^p) and eosin (w^e) in Drosophila melanogaster (102). The order of effect in females is w^ew^e, w^ew, w^ew^p, w^pw^p, w^pw, ww. Treating w as an amorph, w^p appears to be a hypomorph in w^pw^p and w^pw but acts as an antimorph in w^ew^p since this is lighter than w^ew.

Figures 1 to 3 are intended to bring out the relations in a system in which the rate of formation of a product depends jointly on the concentration of a substrate and of an enzyme, during a period in which the former is in flux equilibrium. This is a limiting case in which b is indefinitely large. At least 3 sorts of curves are of interest in this (and other) cases. In figure 1 the product is plotted against concentration of

enzyme (assumed to be determined by a series of alleles). There is approach to the substrate limit according to the relation $\dot{P} = \dot{S}\frac{c(E)}{c(E)+d}$. There is increasing dominance of genes determining higher values of (E). Curves of somewhat this character have been described for vestigial alleles (172) and white alleles (180) in Drosophila and other cases. A type of curve that is of interest in relation to factor interactions is that in which product is plotted against substrate for different dosages of the agent (fig. 2). In the limiting case treated here, each value of the latter has a certain multiplicative effect on the product produced from a given amount of substrate, in agreement with the common cumulative effect of independent series of alleles. A third type of curve is that of product plotted against time, for varying dosages

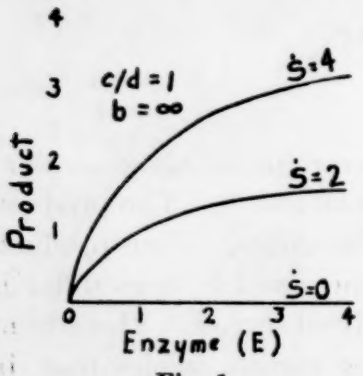


Fig. 1

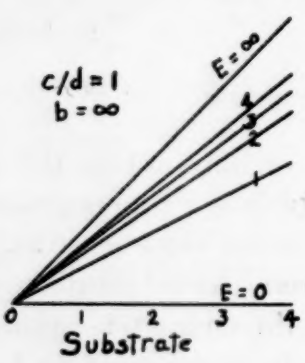


Fig. 2

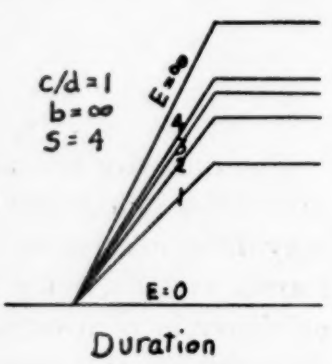


Fig. 3

Figs. 1-3. Quantitative relations in a system in which an observed product depends jointly on amounts of substrate and of a gene controlled enzyme, assuming flux equilibrium. Further description in text.

of the gene controlled agent. The simple form of theory used here gives a linear increase during the reaction. The condition of flux equilibrium cannot however hold near the beginning and end of the reaction. Sigmoid curves such as those described by Ford and Huxley (94, 95) are expected.

Figures 4 to 6 illustrate relations between product and substrate in a chain of reactions, subject to a gene controlled agent (A). Figures 4 and 5 represent cases in which c/bd has intermediate values while figure 6 represents the opposite extreme from figures 1 to 3, that in which the product is determined by substrate or agent, whichever is more limited in amount. By interchanging S and A, the curves may also be used to illustrate relations between product and agent for different substrates. $\dot{A}$ may represent either rate of formation of a noncatalytic factor in the reaction or (if b = 1) the concentration of a catalyst. Curves of the

intermediate type are perhaps more typical of multiple allelic series that act on single characters than either extreme. The ceiling effect, exhibited in extreme form in figure 6, seems to be illustrated by the effects of the albino series in brown guinea pigs (Pbb) as compared with the dark eyed sepias (PB) and even these seem to exhibit considerable ceiling effect compared with the pale browns (ppbb) and pale sepias (ppB).

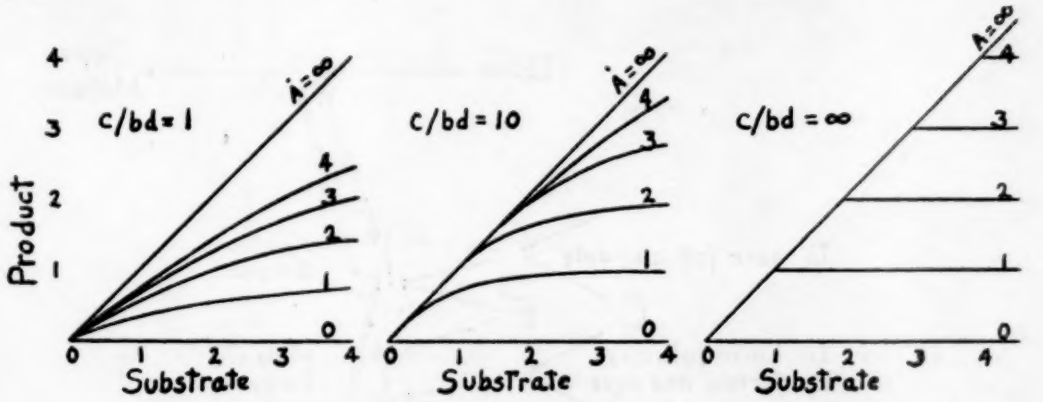


Fig. 4 Fig. 5 Fig. 6

Figs. 4–6. Quantitative relations between substrate and product for different values of the function of rate constants c/bd and different rates (Å) of production of a gene controlled agent. Further description in text.

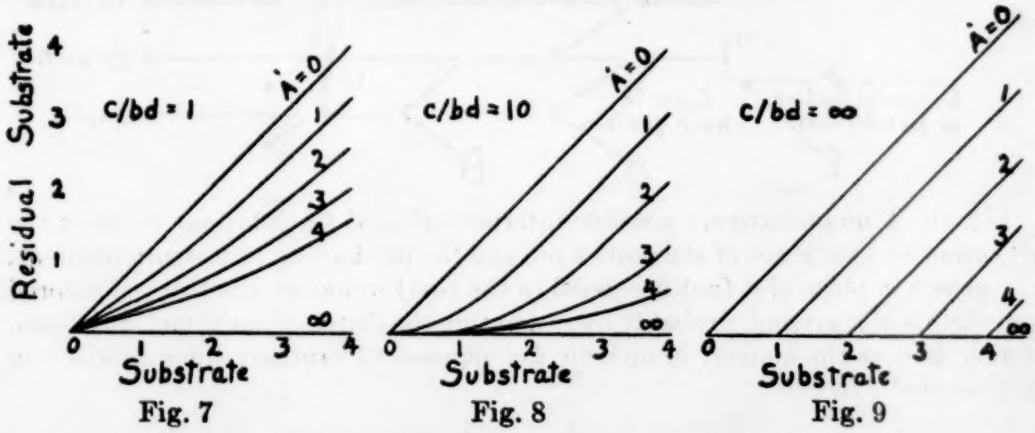


Fig. 7 Fig. 8 Fig. 9

Figs. 7–9. Quantitative relations between rates of production of substrate and of a product other than that due to the gene controlled agent under consideration, which thus behaves as an inhibitor. Further description in text.

Figures 7 to 9 illustrate the relations between rates of production of substrate and of a product (R) other than that due to the agent under consideration. Genes determining the latter thus act as inhibitors of the product in question. Figure 9 represents the extreme case in which increase in the agent has a simple subtractive effect, and incidentally a threshold effect, on the product as related to the substrate. Several

examples of threshold effects may be found in the data in table 1. Figures 7 and 8 illustrate cases intermediate between the subtractive effect (found if c/bd = ∞) and the multiplicative reduction of the product (found if c/bd = 0).

Figure 10 represents a possible system of relations among the color factors of the guinea pig in which the insertion of suitable rate constants according to the above theory gives reasonably good agreement with

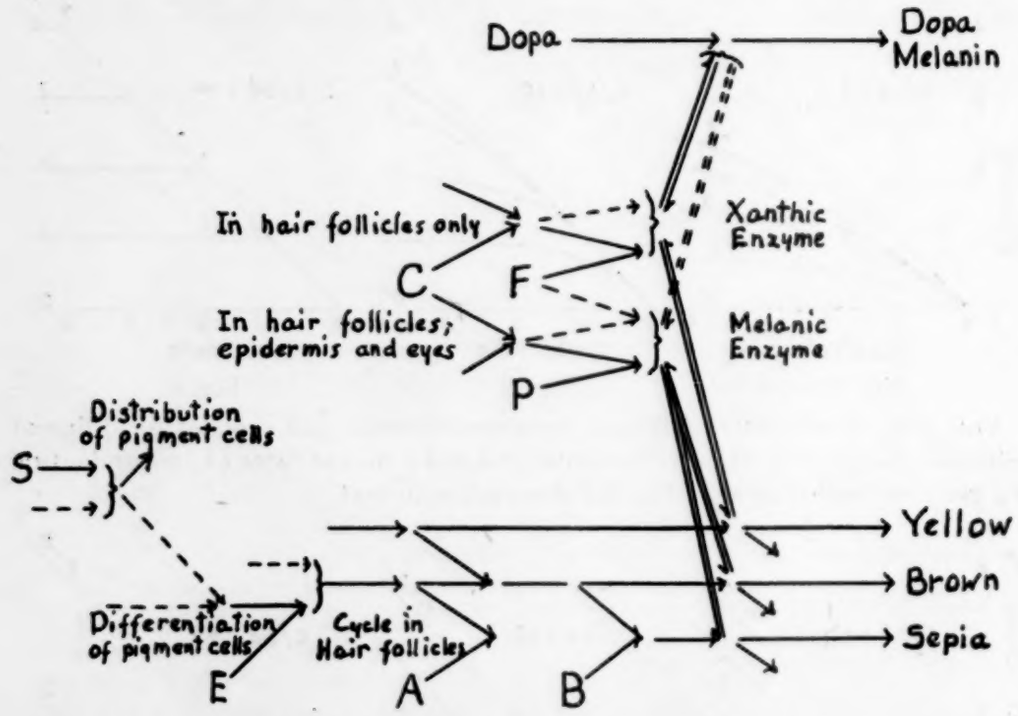


Fig. 10. A quantitatively possible interpretation of the relations between the actions of certain genes of the guinea pig and the production of melanin pigment. The gene s in place of S (not discussed in the text) produces a pattern of colored spots on a white ground, probably by restricting the distribution of melanophores. It also reduces the amount of melanic (as opposed to xanthic) differentiation in tortoise shells ($e^{p}e^{p}$).

the observed quantitative relations in all factor combinations as well as accounting for the dopa reactions. While it is not the only possible scheme, the data impose severe restrictions on hypotheses. There seems little doubt that each gene or its immediate product has a modifying effect on reaction rates at one or more points in a system of chain reactions of this general nature.

Morphogenesis. Among the most interesting problems in physiological genetics is that of the control of developmental patterns by a

system of genes, believed to be distributed without change to every cell in the body. A possible mechanism of such control has been suggested in connection with the discussion of cytoplasmic differentiation. The scope of the present article does not permit review of the extensive literature bearing on morphogenetic action of genes.

In general, the reaction chains connecting primary gene action and observed effects on morphological characters must be longer, more ramifying and more heterogeneous than where effects are on intracellular products. Even more indirect are the relations of genes to modes of behavior of the organism as a whole, although there are cases in which there is simple mendelian heredity. Most indirect of all is genetic control of extraorganic structure (form of spider webs, nests of termites, of wasps, of birds, etc.). The heredity of structures at this level is controlled by hereditary behavior of the organism which is controlled by hereditary structure at the organic level, which in turn is controlled by hereditary cellular behavior and this by elements of cellular constitution tracing perhaps directly to gene products.

REFERENCES

(1) ALEXANDER, J. AND C. B. BRIDGES. Colloid chemistry. New York: The Chemical Catalog Co. **2:** 9-58, 1928.
(2) ANDERSON, E. G. Genetics **9:** 442, 1924.
(3) BALTZER, F. Rev. Suisse Zool. **37:** 325, 1930.
(4) BATESON, W. Mendel's Principles of heredity. Cambridge Univ. Press, 413 pp., 1913.
(5) BAWDEN, F. C. AND N. W. PRICE. Proc. Roy Soc. London (B) **123:** 274, 1937.
(6) BEADLE, G. W. Cornell Agric. Expt. Sta. Memoirs **135:** 3-12, 1931.
(7) BEADLE, G. W. Ztschr. ind. Abst. Ver. **63:** 195, 1932.
(8) BEADLE, G. W. Cytologia **3:** 142, 1932.
(9) BEADLE, G. W. Cytologia **4:** 209, 1933.
(10) BEADLE, G. W. Genetics **37:** 587, 1937.
(11) BEADLE, G. W., R. L. ANDERSON AND J. MAXWELL. Proc. Nat. Acad. Sci. **24:** 80, 1938.
(12) BEADLE, G. W. AND B. EPHRUSSI. Genetics **21:** 225, 1936.
(13) BEADLE, G. W. AND B. EPHRUSSI. Genetics **22:** 76, 1937.
(14) BEADLE, G. W. AND L. W. LAW. Proc. Soc. Exper. Biol. and Med. **37:** 621, 1938.
(15) BELLAMY, A. W. Genetics **13:** 226, 1928.
(16) BELLING, J. J. Genetics **18:** 177, 1927.
(17) BELLING, J. Univ. Calif. Publ. Botany **14:** 307, 1928.
(18) BELLING, J. Genetics **18:** 388, 1933.
(19) BERGER, C. J. Hered. **31:** 2, 1940.
(20) BERNSTEIN, F. Ztschr. ind. Abst. Ver. **37:** 237, 1925.

(21) BITTNER, J. J. Am. J. Cancer **15**: 2202, 1931.
(22) BLAKESLEE, A. F. Proc. Am. Acad. **40**: 205, 1904.
(23) BLAKESLEE, A. F. Proc. Nat. Acad. Sci. **7**: 116, 1921.
(24) BOYCOTT, A. E., C. DIVER, S. L. GARSTANG AND F. M. TURNER. Phil. Trans. Roy. Soc. London (B) **219**: 51, 1930.
(25) BRIDGES, C. B. J. Exper. Zool. **28**: 337, 1919.
(26) BRIDGES, C. B. Proc. Nat. Acad. Sci. **11**: 701, 1925.
(27) BRIDGES, C. B. J. Hered. **29**: 11, 1938.
(28) BRIDGES, C. B. AND P. N. BRIDGES. J. Hered. **30**: 475, 1939.
(29) BRIDGES, C. B. AND M. DEMEREC. Drosophila Information Service no. 9. Carnegie Inst. of Washington. 129 pp., 1938.
(30) BRIDGES, C. B. AND T. H. MORGAN. Carnegie Inst. Washington Publ. 327, 251 pp., 1923.
(31) BRINK, R. A. Quart. Rev. Biol. **4**: 520, 1929.
(32) BRINK, R. A. Am. Nat. **66**: 443, 1932.
(33) CAMMIDGE, P. J. AND H. A. H. HOWARD. J. Genetics **16**: 387, 1926.
(34) CAMMIDGE, P. J. AND H. A. H. HOWARD. Proc. Roy. Soc. Med. **23**: 1341, 1930.
(35) CASPARI, E. Arch. Entw.-mech. **130**: 353, 1933.
(36) CASPARI, E. Ztschr. ind. Abst. Ver. **71**: 546, 1936.
(37) CASPERSSON, T. Skand. Arch. Physiol. **73**: Suppl. 8: 1, 1936.
(38) CASTLE, W. E. J. Genetics **14**: 225, 1924.
(39) CASTLE, W. E. AND C. E. KEELER. Proc. Nat. Acad. Sci. **19**: 92, 1933.
(40) CASTLE, W. E. AND C. E. KEELER. Proc. Nat. Acad. Sci. **19**: 403, 1933.
(41) CATCHESIDE, D. G. J. Genetics **38**: 345, 1939.
(42) CHARLES, D. C. Genetics **23**: 523, 1938.
(43) CHASE, H. B. Genetics **24**: 610, 1938.
(44) CORRENS, C. Ztschr. ind. Abst. Ver. **1**: 291, 1909.
(45) CORRENS, C. Verh. V. Int. Kong. Vererb. (Ztschr. ind. Abst. Ver. Suppl. I): 131, 1928.
(46) CUÉNOT, L. Arch. Zool. Exper. et Gen. (4) 1 Notes et Revues 33, 1903.
(47) DANFORTH, C. H. Proc. Soc. Exper. Biol. and Med. **26**: 86, 1928.
(48) DANFORTH, C. H. Genetics **14**: 256, 1929.
(49) DANFORTH, C. H. AND F. FOSTER. J. Exper. Zool. **52**: 443, 1929.
(50) DANNEEL, R. AND K. SCHAUMANN. Biol. Zbl. **58**: 242, 1938.
(51) DE HAAN, H. Bibl. Genet. **10**: 358, 1933.
(52) DEMEREC, M. Proc. Nat. Acad. Sci. **15**: 834, 1929.
(53) DEMEREC, M. Proc. Nat. Acad. Sci. **20**: 354, 1934.
(54) DEMEREC, M. Cold Spring Harbor Symposia on Quant. Biol. **2**: 110, 1934.
(55) DEMEREC, M. Proc. Nat. Acad. Sci. **22**: 350, 1936.
(56) DEMEREC, M. Genetics **22**: 469, 1937.
(57) DEMEREC, M. AND M. H. HOOVER. Genetics **24**: 271, 1939.
(58) DEMEREC, M. AND H. SLIZYNSKA. Genetics **22**: 641, 1937.
(59) DETLEFSEN, J. A. Carnegie Inst. Washington Publ. **205**: 1, 1914.
(60) DIVER, C. AND I. ANDERSSON-KOTTÖ. J. Genetics **35**: 447, 1938.
(61) DOBZHANSKY, T. Biol. Rev. **11**: 364, 1936.
(62) DORRIS, F. Arch. Entw.-mech. **138**: 323, 1938.
(63) DORRIS, F. J. Exper. Zool. **80**: 315, 1939.

(64) DUBININ, N. P. J. Genetics **26:** 37, 1932.
(65) DUBININ, N. P. AND B. W. SIDOROFF. Am. Nat. **68:** 377, 1934.
(66) DUBININ, N. P. AND B. W. SIDOROFF. J. de Biol. (U.S.S.R.) **4:** 555, 1935.
(67) DUGGAR, B. M. AND J. K. ARMSTRONG. Ann. Missouri Bot. Gard. **10:** 191, 1923.
(68) DUNN, L. C. Proc. Nat. Acad. Sci. **14:** 816, 1928.
(69) DUNN, L. C. Nature **129:** 130, 1932.
(70) DUNN, L. C. J. Genetics **29:** 317, 1934.
(71) DUNN, L. C. Proc. Nat. Acad. Sci. **23:** 474, 1937.
(72) DUNN, L. C. AND W. EINSELE. J. Genetics **36:** 145, 1938.
(73) DUNN, L. C. AND S. GLUECKSOHN-SCHOENHEIMER. Genetics **24:** 589, 1939.
(74) DURHAM, F. M. Proc. Roy. Soc. London **74:** 310, 1904.
(75) DUSHANE, G. P. J. Exper. Zool. **72:** 1, 1935.
(76) DUSHANE, G. P. J. Exper. Zool. **78:** 485, 1938.
(77) EAST, E. M. Bibl. Genetica **5:** 331, 1929.
(78) EAST, E. M. Proc. Nat. Acad. Sci. **20:** 364, 1934.
(79) EAST, E. M. Am. Nat. **68:** 289, 402, 1934.
(80) EASTLICK, H. L. J. Exper. Zool. **82:** 131, 1939.
(81) ELLENHORN, J., A. PROKOFYEVA AND H. J. MULLER. C. R. Acad. de Sciences de l'U.R.S.S. **1:** 234, 1935.
(82) EMERSON, R. A. Genetics **14:** 488, 1929.
(83) EMERSON, S. Genetics **23:** 190, 1938.
(84) EPHRUSSI, B. Am. Nat. **72:** 5, 1938.
(85) EPHRUSSI, B. AND G. W. BEADLE. J. Genetics **33:** 407, 1936.
(86) EPHRUSSI, B. AND G. W. BEADLE. Genetics **22:** 65, 1937.
(87) EPHRUSSI, B. AND G. W. BEADLE. Genetics **22:** 479, 1937.
(88) EPHRUSSI, B. AND G. W. BEADLE. Bull. Biologique **71:** 54, 75, 1937.
(89) EPHRUSSI, B. AND S. CHEVAIS. Bull. Biologique **72:** 48, 1938.
(90) EPHRUSSI, B. AND S. CHEVAIS. Proc. Nat. Acad. Sci. **23:** 428, 1938.
(91) FISHER, R. A. Am. Nat. **62:** 115, 1928.
(92) FISHER, R. A. Am. Nat. **62:** 571, 1928.
(93) FISHER, R. A. Biol. Rev. **6:** 345, 1931.
(94) FORD, E. B. AND J. S. HUXLEY. Brit. J. Exper. Biol. **5:** 112, 1927.
(95) FORD, E. B. AND J. S. HUXLEY. Arch. Entw.-mech. **117:** 67, 1929.
(96) GARROD, A. E. Inborn errors of metabolism. Oxford Med. Publ. 216 pp., 1923.
(97) GOLDSCHMIDT, R. Physiologische Theorie der Vererbung. Berlin, J. Springer, 1927.
(98) GOLDSCHMIDT, R. Bibl. Genetica **11:** 1, 1933.
(99) GOLDSCHMIDT, R. Am. Nat. **68:** 5, 1934.
(100) GOLDSCHMIDT, R. Ztschr. ind. Abst. Ver. **67:** 1, 1935.
(101) GOLDSCHMIDT, R. Physiological genetics. New York, McGraw-Hill Book Co., 375 pp., 1938.
(102) GOLDWEBER, S. Am. Nat. **73:** 568, 1939.
(103) GOODSPEED, T. H. AND R. E. CLAUSEN. Am. Nat. **51:** 31, 92, 1917.
(104) GORDON, M. AND A. C. FRASER. J. Hered. **22:** 168, 1931.
(105) GORTNER, R. A. Outlines of biochemistry. John Wiley & Sons, New York, 1017 pp., 1938.

(106) GOWEN, J. W. J. Exper. Zool. **65**: 83, 1933.
(107) GOWEN, J. W. Genetics **19**: 89, 1934.
(108) GOWEN, J. W. Proc. Nat. Acad. Sci. **26**: 8, 1940.
(109) GOWEN, J. W. AND E. H. GAY. Genetics **18**: 1, 1933.
(110) GRÜNEBERG, H. J. Genetics **34**: 169, 1937.
(111) GULICK, A. Quart. Rev. Biol. **13**: 1, 140, 1938.
(112) HADORN, E. Verh. deutsch. Zool. Gesell. 97, 1936.
(113) HADORN, E. Arch. Entw.-mech. **136**: 400, 1937.
(114) HADORN, E. Proc. Nat. Acad. Sci. **23**: 478, 1937.
(115) HADORN, E. AND J. NEEL. Arch. Entw.-mech. **138**: 281, 1938.
(116) HAEMMERLING, J. Arch. Entw.-mech. **131**: 1, 1934.
(117) HAEMMERLING, J. Arch. Entw.-mech. **132**: 424, 1934.
(118) HALDANE, J. B. S. Am. Nat. **64**: 87, 1930.
(119) HALDANE, J. B. S. The Lancet, December 24, 1938.
(120) HALDANE, J. B. S. J. Genetics **37**: 365, 1939.
(121) HAMBURGER, V. J. Exper. Zool. **70**: 43, 1935.
(122) HAMBURGER, V. J. Exper. Zool. **73**: 319, 1936.
(123) HANNA, W. F. Ann. Bot. **39**: 431, 1925.
(124) HANSON, F. B. Physiol. Rev. **13**: 466, 1933.
(125) HARLAND, S. C. J. Genetics **28**: 315, 1934.
(126) HARVEY, E. B. Science **82**: 277, 1935.
(127) HAWKINS, L. E. Genetics **16**: 547, 1931.
(128) HEIDENTHAL, G. Genetics **25**: 197, 1940.
(129) HEITZ, E. AND H. BAUER. Ztschr. Zellf. **17**: 67, 1933.
(130) HUXLEY, J. S. AND G. R. DE BEER. The elements of experimental embryology. Cambridge: The University Press. 514 pp.
(131) IRWIN, M. R. J. Genetics **35**: 351, 1938.
(132) IRWIN, M. R. Genetics **24**: 709, 1939.
(133) IRWIN, M. R. AND L. J. COLE. J. Exper. Zool. **73**: 85, 1936.
(134) IRWIN, M. R. AND L. J. COLE. J. Exper. Zool. **73**: 309, 1936.
(135) IRWIN, M. R., L. J. COLE AND C. D. GORDON. J. Exper. Zool. **73**: 285, 1936.
(136) JOHNSON, I. J. AND E. S. MILLER. Cereal Chem. **15**: 345, 1938.
(137) JOLLOS, V. Arch. f. Protistenkunde **83**: 197, 1934.
(138) JOLLOS, V. Biol. Zentralbl. **35**: 390, 1935.
(139) KAUFMANN, B. B. J. Morphol. and Physiol. **56**: 125, 1934.
(140) KOLLER, P. J. Genetics **22**: 103, 1930.
(141) KOLTZOFF, N. K. Biol. Zbl. **48**: 345, 1928.
(142) KOSSEL, A. The protamines and histones. London: Longmans Green & Co., 107 pp., 1928.
(143) KRÖNING, F. Arch. Entw.-mech. **121**: 470, 1930.
(144) KÜHN, A. Nach. d. Gesellsch. Wiss. z. Göttingen Math. and Phys. Kl. **1927**: 407, 1927.
(145) KÜHN, A. Ztschr. ind. Abst. Ver. **73**: 419, 1937.
(146) KÜHN, A., E. CASPARI AND E. PLAGGE. Ges. Wiss. Göttingen. Nach. Biol. **2**: 1, 1935.
(147) LANDSTEINER, K. The specificity of serological reactions. Baltimore, Md., Charles C. Thomas, 178 pp., 1936.
(148) LANDSTEINER, K. AND P. LEVINE. J. Exper. Med. **48**: 731, 1928.
(149) LAWRENCE, W. J. C. AND J. R. PRICE. Biol. Rev. **15**: 35, 1940.

(150) LAWRENCE, W. J. C. AND R. SCOTT-MONCRIEFF. J. Genetics **30**: 156, 1935.
(151) LI, J. C. Genetics **12**: 1, 1927.
(152) LINDEGREN, C. C. Zbl. f. Bakt., Paras. u. Infektionskrankheiten **93**: 389, 1936.
(153) LINDEGREN, C. C. AND S. RUMANN. J. Genetics **36**: 395, 1938.
(154) LITTLE, C. C. J. Cancer Res. **8**: 75, 1924.
(155) LITTLE, C. C. AND B. W. JOHNSON. Proc. Soc. Exper. Biol. and Med. **19**: 163, 1922.
(156) LITTLE, C. C. AND L. C. STRONG. J. Exper. Zool. **41**: 93, 1924.
(157) LITTLE, C. C. AND E. E. TYZZER. J. Med. Res. **33**: 393, 1916.
(158) LOEB, L. Biol. Bull. **40**: 143, 1921.
(159) LOEB, L. AND H. D. KING. Arch. Path. **12**: 203, 1931.
(160) LOEB, L. AND S. WRIGHT. Am. J. Path. **3**: 251, 1927.
(161) MCCLINTOCK, B. Ztschr. f. Zellf. u. Mikr. Anat. **19**: 191, 1933.
(162) MACDOWELL, E. C. AND M. N. RICHTER. Biol. Zentralbl. **52**: 266, 1932.
(163) MCKINNEY, H. H. J. Hered. **28**: 51, 1937.
(164) MAINX, F. Ztschr. ind. Abst. Ver. **75**: 256, 1938.
(165) MANGELSDORF, P. C. AND G. S. FRAPS. Science **73**: 241, 1931.
(166) MATSUMURA, S. Arch. Zoologico **16**: 1270, 1930.
(167) MATSUMURA, S. Nagaio Sericult. Expt. Sta. Bull. 28, 1934.
(168) MAZIA, D. AND L. JAEGER. Proc. Nat. Acad. Sci. **25**: 456, 1939.
(169) MENDEL, G. Translation in Bateson's Mendel's Principles of heredity. 1913. Cambridge Univ. Press (p. 335-386), 1866.
(170) METZ, C. W. Am. Nat. **73**: 457, 1939.
(171) MOHR, O. L. Ztschr. ind. Abst. Ver. **50**: 113, 1929.
(172) MOHR, O. L. Proc. 6th Int. Cong. Genet. **1**: 190, 1932.
(173) MORGAN, T. H. Proc. Roy. Soc. London B **94**: 162, 1922.
(174) MORGAN, T. H. Am. Nat. **60**: 489, 1926.
(175) MORGAN, T. H. AND C. B. BRIDGES. J. Exper. Zool. **15**: 429, 1913.
(176) MULLER, H. J. Proc. Int. Cong. Plant Sciences **1**: 897, 1929.
(177) MULLER, H. J. J. Genetics **22**: 299, 1930.
(178) MULLER, H. J. Proc. 6th Int. Cong. Genet. **1**: 213, 1932.
(179) MULLER, H. J. The science of radiology. Chapter 17: 305, 1934.
(180) MULLER, H. J. J. Genetics **30**: 407, 1935.
(181) MULLER, H. J. Am. Nat. **69**: 405, 1935.
(182) MULLER. H. J. Scientific Monthly **44**: 210, 1937.
(183) MULLER, H. J., B. B. LEAGUE AND C. A. OFFERMANN. Anat. Rec. **51**: 110, 1931.
(184) MULLER, H. J. AND A. PROKOFYEVA. Proc. Nat. Acad. Sci. **21**: 16, 1935.
(185) NABOURS, R. K. Bibl. Genetica **5**: 27, 1929.
(186) NABOURS, R. K. Proc. Nat. Acad. Sci. **16**: 350, 1930.
(187) OFFERMANN, C. A. Bull. Acad. Sci. U.S.S.R. Cl. Sci. Math. Nat. **1**: 129, 1935.
(188) OLIVER, C. P. Quart. Rev. Biol. **9**: 381, 1934.
(189) ONSLOW, H. Proc. Roy. Soc. London B **89**: 36, 1915.
(190) ONSLOW, H. Biochem. J. **17**: 334, 564, 1923.
(191) ONSLOW, M. W. Anthocyanin pigments of plants. Cambridge Univ. Press, 318 pp., 1916.
(192) PAINTER, T. S. Genetics **19**: 448, 1934.

(193) PAINTER, T. S. Am. Nat. **63**: 315, 1939.
(194) PANSHIN, J. B. C. R. Acad. Sci. U.S.S.R. **1935**: 85, 1935.
(195) PATTERSON, T. J., W. S. STONE, S. BEDICHEK AND M. SUCHE. Am. Nat. **68**: 359, 1934.
(196) PEARL, R. J. Washington Acad. Sci. **25**: 253, 1935.
(197) PEASE, M. S. Verh. V. Int. Kong. Ver., Ztschr. ind. Abst. Ver. Suppl. **2**: 1153, 1927.
(198) PENROSE, L. S. The Lancet **1**: 23, 1935.
(199) PENROSE, L. S. The Lancet **2**: 192, 1935.
(200) PINCUS, G. J. Exper. Zool. **32**: 439, 1929.
(201) PLOUGH, H. H. AND P. T. IVES. Genetics **20**: 42, 1935.
(202) PLUNKETT, C. R. Proc. 6th Int. Cong. Genetics **2**: 160, 1932.
(203) PUNNETT, R. C. J. Genetics **23**: 265, 1930.
(204) PUGH, C. E. M. Biochem. J. **27**: 475, 1933.
(205) REDFIELD, H. Genetics **11**: 482, 1926.
(206) REED, S. C. J. Exper. Zool. **79**: 331, 337, 1938.
(207) REED, S. C. AND G. SANDER. Growth **1937**: 194, 1938.
(208) RENNER, O. Biol. Zbl. **44**: 309, 1924.
(209) RENNER, O. Bibl. Genetica **9**: 1, 1925.
(210) RHOADES, M. M. J. Genetics **27**: 71, 1933.
(211) RHOADES, M. M. Genetics **23**: 377, 1938.
(212) RICHTER, M. M. AND E. C. MACDOWELL. Physiol. Rev. **15**: 509, 1935.
(213) RUSSELL, E. S. Genetics **24**: 332, 1939.
(214) RUSSELL, W. L. Genetics **24**: 645, 1939.
(215) SCHARRER, B. AND E. HADORN. Proc. Nat. Acad. Sci. **24**: 236, 1938.
(216) SCHULTZ, J. Am. Nat. **69**: 30, 1935.
(217) SCHULTZ, J. Proc. Nat. Acad. Sci. **22**: 27, 1936.
(218) SCHULTZ, J. Biological effects of radiation. pp. 1209–1262. McGraw Hill Book Co., New York, 1936.
(219) SCHULTZ, W. Arch. Entw.-mech. **41**: 535, 1915.
(220) SCHULTZ, W. Arch. Entw.-mech. **105**: 677, 1925.
(221) SCHULTZ, W. Schriften d. Phys. Öken. Gesell, Königsberg **67**: 1, 1930.
(222) SCHUURMANN, J. J. Genetica **19**: 273, 1937.
(223) SCOTT-MONCRIEFF, R. J. Genetics **32**: 117, 1936.
(224) SEXTON, E. W. AND A. R. CLARK. Nature **117**: 194, 1926.
(225) SEXTON, E. W. AND C. F. A. PANTEN. **119**: 119, 1927.
(226) SILOW, R. A. J. Genetics **38**: 229, 1939.
(227) SIRKS, M. J. Proc. Kon. Akad. Wetenschap. Amsterdam. **34**: 1057, 1164, 1340, 1931.
(228) SIRKS, M. J. Biol. Rev. **4**: 113, 1938.
(229) SMITH, P. E. AND E. C. MACDOWELL. Anat. Rec. **46**: 249, 1930.
(230) STANLEY, W. M. Physiol. Rev. **19**: 524, 1939.
(231) STERN, C. Biol. Zentralbl. **49**: 261, 1929.
(232) STERN, C. Multiple allelie. Handb. d. Vererb. Wiss. **1**: 1, 1930.
(233) STERN, C. Genetics **21**: 625, 1936.
(234) STERN, C. Am. Nat. **72**: 350, 1938.
(235) STRONG, L. C. J. Cancer Res. **13**: 103, 1929.
(236) STURTEVANT, A. H. Proc. Soc. Exper. Biol. and Med. **17**: 70, 1920.

(237) STURTEVANT, A. H. Proc. Nat. Acad. Sci. **7**: 235, 1921.
(238) STURTEVANT, A. H. Science **58**: 269, 1923.
(239) STURTEVANT, A. H. Genetics **10**: 117, 1925.
(240) STURTEVANT, A. H. Ztschr. Wiss. Zool. **135**: 323, 1929.
(241) STURTEVANT, A. H. Carnegie Inst. Washington Publ. 399, 1 1929.
(242) TANAKA, Y. Genetics **9**: 479, 1924.
(243) THIMANN, K. V. AND G. W. BEADLE. Proc. Nat. Acad. Sci. **23**: 143, 1937.
(244) TIMOFEEFF-RESSOVSKY, N. W. Ergebn. med. Strahlenforsch. **5**: 131, 1931.
(245) TIMOFEEFF-RESSOVSKY, N. W. Biol. Rev. **9**: 411, 1934.
(246) TIMOFEEFF-RESSOVSKY, N. W. Die Naturwiss. (1935) 494, 1935.
(247) TIMOFEEFF-RESSOVSKY, N. W. AND K. G. ZIMMER. Strahlentherapie **66**: 684, 1939.
(248) TIMOFEEFF-RESSOVSKY, N. W., K. G. ZIMMER AND M. DELBRÜCK. Nachrt. Ges. Wiss. Göttingen NF 1 189, 1935.
(249) TODD, C. Proc. Roy. Soc. London B. **106**: 20, 1930.
(250) TODD, C. Ibid. B **107**: 197, 1931.
(251) TOYAMA, K. J. Genetics, **8**: 322, 1913.
(252) TRIMBLE, H. C. AND C. E. KEELER. J. Hered. **29**: 281, 1938.
(253) TROLAND, L. T. Am. Nat. **51**: 321, 1917.
(254) WAALER, G. H. M. Ztschr. ind. Abst. Ver. **45**: 279, 1927.
(255) WADDINGTON, C. H. Am. Nat. **73**: 300, 1939.
(256) WADDINGTON, C. H. An introduction to modern genetics. New York: The MacMillan Co., 441 pp., 1939.
(257) WELLS, H. G. Arch. Pathol. **9**: 1044, 1930.
(258) WETTSTEIN, F. VON. Bibliotheca Genetica **10**: 1, 1928.
(259) WETTSTEIN, F. VON. Wiss. Woche Frankfurt/M. Erbbiologie **1**: 31, 1934.
(260) WHITING, A. R. J. Genetics **29**: 99, 1934.
(261) WHITING, P. W. Biol. Bull. **63**: 296, 1932.
(262) WIENER, A. Blood groups and blood transfusion. Baltimore, C. C. Thomas, 220 pp., 1940.
(263) WILLIER, B. H. Sex and internal secretions. (2nd ed. Edited by E. Allen). Baltimore: Williams & Wilkins. Chapter 3: 6–144, 1939.
(264) WINGE, O. J. Genetics **34**: 81, 1937.
(265) WIT, W. Genetica **19**: 1, 1937.
(266) WRIGHT, S. Carnegie Inst. Washington Publ. **241**: 59, 1916.
(267) WRIGHT, S. J. Hered. **8**: 224, 1917.
(268) WRIGHT, S. Genetics **10**: 223, 1925.
(269) WRIGHT, S. Genetics **12**: 530, 1927.
(270) WRIGHT, S. Am. Nat. **63**: 274, 556, 1929.
(271) WRIGHT, S. Am. Nat. **66**: 282, 1932.
(272) WRIGHT, S. Am. Nat. **68**: 25, 1934.
(273) WRIGHT, S. Proc. 7th Int. Gen. Cong., 1941: 319–327.
(274) WRIGHT, S. AND O. N. EATON. Genetics **11**: 333, 1926.